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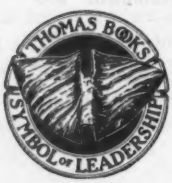

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THE NONSPECIFIC FLUORESCENT MATERIAL IN ESTROGEN EXTRACTS FROM THE URINE OF PREGNANT WOMEN*

CLAUDE J. MIGEON, M.D., W. R. SLAUNWHITE, JR., PH.D.,† R. ALDOUS,‡ R. FOX,‡ R. HARDY,‡ D. JOHNSON‡ AND W. PERKINS‡

The Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City, Utah

INTRODUCTION

URINARY estrogen extracts are known to contain some nonspecific material which can 1) induce the Kober reaction, or 2) fluoresce following the addition of acid. Using the countercurrent technique of fractionation, the fractions resembling estrone, estradiol and estriol have been found to represent only 30 per cent of the total fluorescence in urinary extracts from normal adults and 70 to 75 per cent during pregnancy (1).

Although the nonspecific material may bear no relation to the estrogenic hormones, there is the possibility that it is made up of compounds which are either hormones themselves or intermediary metabolites of estrogens. Such a possibility has been suggested by the work of Lieberman *et al.* (2), Zondek and Finkelstein (3) and Slaunwhite (4).

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† Damon Runyon Research Fellow. Present address: Roswell Park Memorial Institute, Buffalo, N. Y.

‡ First-year medical students.

In an attempt to characterize this nonspecific fluorescent material, we previously reported the separation of two fractions designated as "E_4" and "E_5" (1). It is the purpose of the present paper to describe certain of the properties of "Fraction E_4" as separated from the urine of pregnant women.

METHODS

Urine collection

Twenty-four-hour urine specimens were collected in bottles containing 15 ml. of 25 per cent acetic acid and stored under refrigeration. An eight-day collection was made on a 28-year-old female in the eighth month of pregnancy.

Extraction procedure

The method of hydrolysis and extraction described by Engel and colleagues (5) was followed, with one exception, *viz.*, the 1 N NaOH extract was brought to pH 6–7 instead of pH 9.

Countercurrent distributions

These were carried out in the 25-tube stainless-steel instrument described by Craig and Post (6). The technique is described elsewhere (7). The following solvent systems were used:

System I—methanol:water (50:50)/carbon tetrachloride.

System II—cyclohexane:ethyl acetate (50:50)/ethanol:water (33:67). Following distribution, the total contents of each tube were pipetted into a separate flask and evaporated to dryness.

Photofluorometric analysis and construction of curves

The technique is described elsewhere (1).

Biologic assay

The mouse uterine-weight method of Evans *et al.* (8) was modified slightly, as previously described (9). Emmens' modification of the Allen and Doisy test was also used (10).

Ultraviolet spectrum studies

Studies were made on alcoholic extracts with the Cary spectrophotometer between the wave lengths of 220 and 320 mμ.

Zimmermann reaction

The dry extract was dissolved in 0.2 ml. of absolute ethanol; then 0.2 ml. of 2.5 N KOH in absolute alcohol and 0.2 ml. of 1 per cent *m*-dinitrobenzene were added. After shaking, the mixture was incubated at 25° C. in the dark for ninety minutes. A 3-ml. portion of absolute ethanol was then added, and the optical density of the mixture was measured on a Beckman DU spectrophotometer at wave lengths of 440, 520 and 600 mμ.

Paper chromatography

The extract was transferred onto a Whatman No. 2 paper strip and concentrated on the origin, according to the method of Bush (11). It was equilibrated overnight and

then run for three hours in the exact manner described by Bush (11). The two following systems of solvents were used:

Benzene:light petroleum (33:66)/methanol:water (80:20).

Benzene/methanol:water (55:45).

The detection of steroids on the chromatogram was carried out by the method of F. L. Mitchell (12).

RESULTS

Countercurrent distribution of the eight-day urinary extract

Using the system of solvents, methanol:water (50:50)/carbon tetrachloride, Curve No. 1 was obtained (Fig. 1). By further distributions, as previously described (1), it was found that 800 μg. of estrone, 320 μg. of estradiol-17β and 8,050 μg. of estriol were excreted per twenty-four hours. The specific fluorescence due to estrone, estradiol and estriol represented 69 per cent of the total fluorescence.

Paper chromatography of the fractions from countercurent distribution

Aliquots representing 1/15 to 1/600 of the contents of each tube of the countercurrent distribution were kept for paper chromatography. Aliquots of tubes numbered 0, 2, 4, 6, 8, 10, 12, and 14 were run in the system benzene:light petroleum (33:66)/methanol:water (80:20). Aliquots of tubes numbered 16, 18, 20, 21, 22, 23 and 24 were run in the system benzene/methanol:water (55:45). The results after color development are shown on the strips in Figure 1. Spots of the same R_f value as pure estrone (E_1) were present on strips 2 to 12. Their distribution corresponded approximately to that found in the countercurrent distribution. This was also true for estradiol (E_2) and estriol (E_3). In addition to spots corresponding to the three natural estrogens, several other spots were found. One had an R_f value close to that of estrone. Three different spots had R_f values somewhat lower than that of estradiol. Seven or eight spots were found in the proximity of estriol (Fig. 1).

Zimmermann reactions of the tube contents from countercurrent distribution

Aliquots of each tube (1/5 to 1/20 of the total) from the first countercurrent distribution (Curve No. 1) were used for the Zimmermann reaction. The reaction was positive only in tubes 4 to 9. Compared to an estrone standard, the amount of reactant in these tubes represented about 80 per cent of the amount of estrone expected from Curve No. 1.

Separation and study of "Fraction E_4"

A series of countercurrent distributions was carried out in order to separate "Fraction E_4" as described in a previous paper (1). The results are shown in Curves No. 2 to No. 4 (Fig. 2). The contents of tubes 15

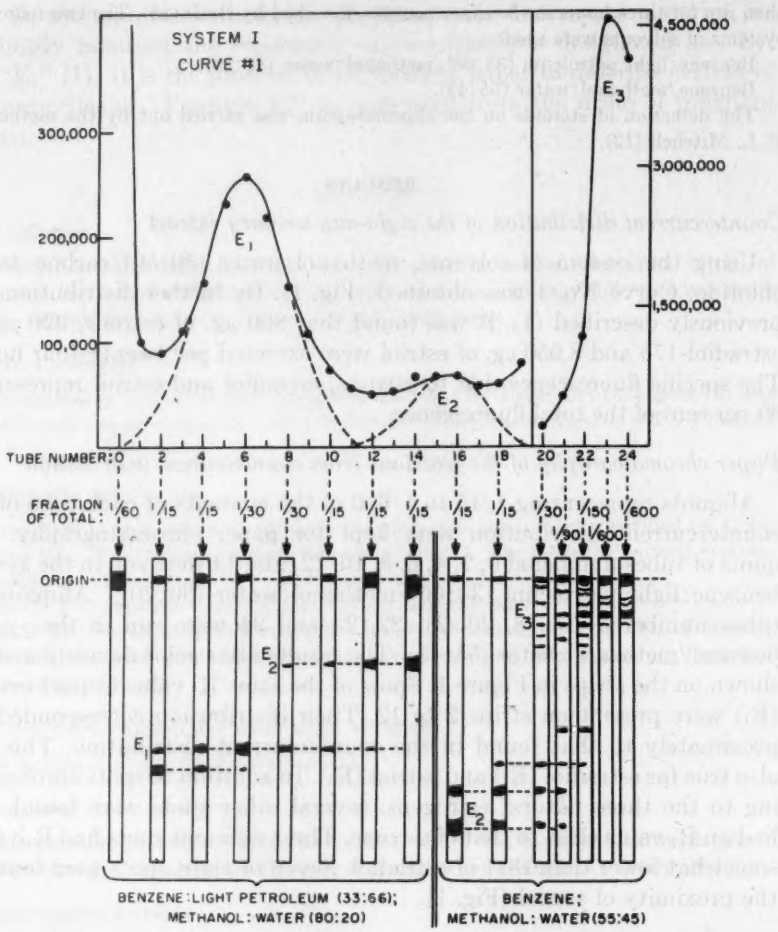

FIG. 1. *Fractionation of an eight-day estrogen extract from the urine of a woman in the eighth month of pregnancy.* Curve No. 1 shows the countercurrent distribution of the extract. Diagrams of paper chromatograms correspond to the indicated fraction of the contents of the corresponding tube in the countercurrent distribution—(E_1) = estrone; (E_2) = estradiol; (E_3) = estriol.

to 23 of Curve No. 4 were pooled. This fraction was tested as follows:

The partition coefficient of "E_4," determined by countercurrent distribution and measurement of fluorescence, was found to be 5 ± 0.5 in System I and 5 ± 0.2 in System II.

The Zimmermann reaction was negative, with an aliquot representing 1/10 of the total.

A bioassay was carried out, using the mouse uterine-weight method. The

assay was set up according to the statistical design of Bliss (13), estriol being used as standard. Assuming that "E_4" had the same fluorescence potency as estriol, "E_4" would be more than 3.16 times as biologically potent as estriol.

An aliquot of "E_4" (1/200 of total "E_4" per ml. of ethanol) was studied in ultraviolet light. The curve is shown in Figure 3 and is compared to that of estrone (50 μg. per ml. and 200 μg. per ml.). A peak was observed at 282 mμ., and a second minor one at 288 mμ. These results are very similar to

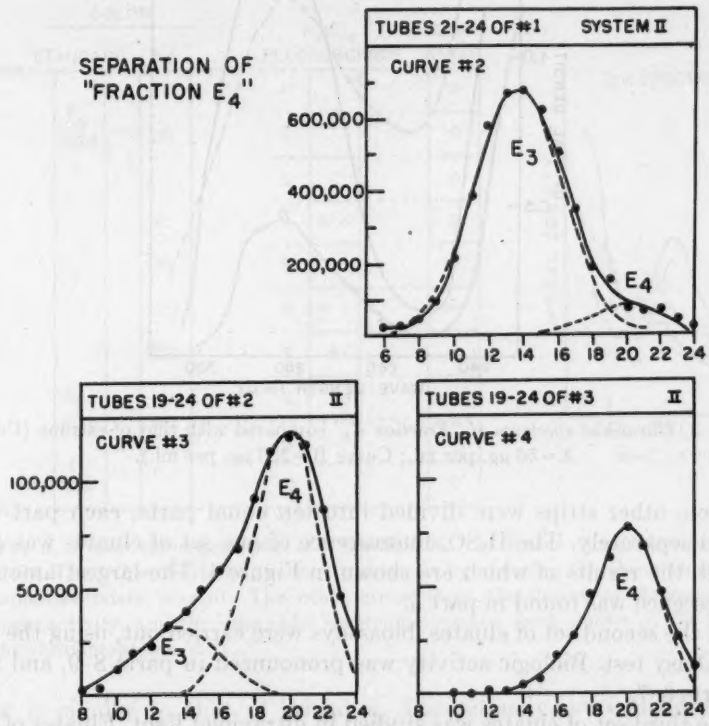

FIG. 2. *Separation of "Fraction E_4" by countercurrent distribution.* By serial distributions of tubes 19–24, it was possible to separate "E_4" from "E_3" in Curve No. 4.

those obtained with the three natural estrogens; however, the minimum of absorption was found at 254 mμ instead of 247 mμ for estrone.

Paper chromatography of "Fraction E_4"

Several aliquots of "Fraction E_4" were chromatographed in the system benzene/methanol:water (55:45).

One paper strip was colored with phosphomolybdate reagent. Four

major spots were found; one was almost on the origin, and the other three had R_f values of approximately 0.58, 0.66 and 0.88. Moreover, two other much lighter spots with R_f values 0.35 and 0.45, respectively, were also present (Fig. 4).

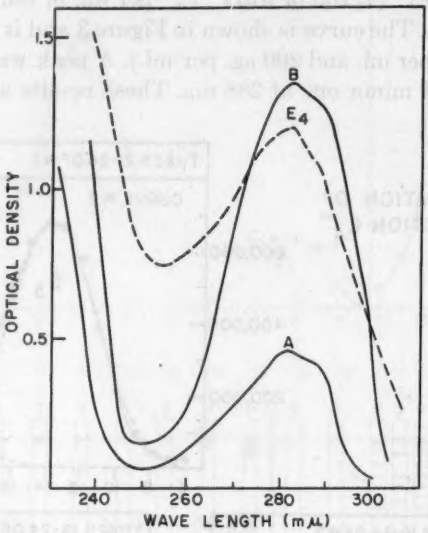

FIG. 3. *Ultraviolet spectrum of "Fraction E_4"* compared with that of estrone (Curve A = 50 μg. per ml.; Curve B = 200 μg. per ml.).

Three other strips were divided into ten equal parts, each part being eluted separately. The H_2SO_4 fluorescence of one set of eluates was determined, the results of which are shown in Figure 4. The largest amount of fluorescence was found in part 5.

On the second set of eluates, bioassays were carried out, using the Allen and Doisy test. Biologic activity was pronounced in parts 8–9, and slight in parts 6–7.

The third set of eluates was studied in ultraviolet light. Eluates of parts 1 and 5 did not show any curve between the wave lengths of 220 and 300 mμ, but curves were obtained with eluates 2, 6 and 7; these curves, however, were somewhat different from those of the three natural estrogens (Fig. 4).

DISCUSSION

Paper chromatography revealed the presence of more than one substance in the contents of each tube of the countercurrent distribution of a urinary extract. This observation demonstrates that countercurrent distribution

separates only groups of substances of similar polarity and can therefore be used only as a first step for further fractionation.

"Fraction E_4" was found to have a biologic activity at least three times that of the estriol standard. Such a determination, however, was made on the assumption that "E_4" and estriol had the same fluorescence potency, an assumption which might have been completely erroneous. Furthermore, the fact that most of the biologic activity was found in the eluate of parts 8–9 of the paper strip (Fig. 4) must be considered. The R_f value of this

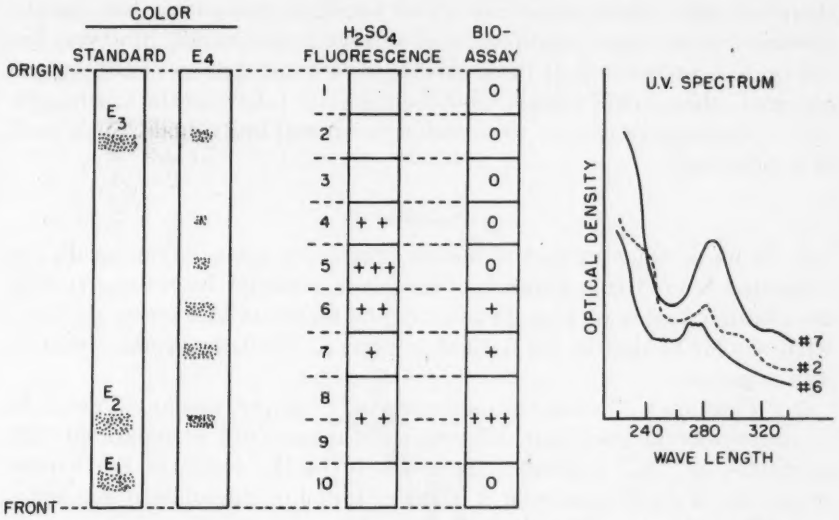

FIG. 4. *Paper chromatography of "Fraction E_4" in system benzene/methanol:water* (55:45). The left strip shows the result obtained by spraying the chromatogram with phosphomolybdate reagent. The other strips show the degree of fluorescence, the biologic activity, and the ultraviolet spectrum obtained from eluates of the ten parts of the chromatograms of "E_4."

spot is similar to that of estradiol. Theoretically, very little estradiol should be present in tubes 21–24 of Curve No. 1 (approximately 2 per cent of the total amount, or 5 μg.); but a small spot corresponding to estradiol was found on the paper strip of tube 21 (Fig. 1). In further separation of "Fraction E_4" from estriol (Fig. 2), estradiol would be carried through the entire fractionation. The biologic activity of total "Fraction E_4" and of parts 8–9 of the paper strip of "E_4" was therefore due to some estradiol contamination. However, the spot of parts 8–9 of the paper strip of "E_4" may have been composed principally of some unknown compound contaminated with a small amount of estradiol. The limit of detection with

phosophomolybdate reagent is approximately 1 microgram per square centimeter, and approximately ten times that amount is necessary to produce a major spot. Hence the color may be due only in part to the estradiol present.

It is evident that several fluorescent substances can be separated from the estrogen extract of human pregnancy urine, their R_f values being definitely different from those of the three natural estrogens. These substances would account for the higher values found by fluorescence analysis than by bioassays. They may be products of estrogen metabolism and therefore more representative of actual estrogen production than are the biologic values. Their relationship to estrogenic hormones, however, has not been demonstrated. If these substances are metabolites of the natural estrogens, they should exhibit some radioactivity following the administration of radioactive estrone or estradiol to normal individuals. Such work is in progress.

CONCLUSIONS

1. In an estrogen extract of human pregnancy urine, "Fraction E_4" as separated from the nonspecific fluorescent material by countercurrent distribution, had some biologic activity and an ultraviolet spectrum somewhat similar to that of the natural estrogens. The Zimmermann reaction was negative.

2. "Fraction E_4," when chromatographed on paper, was found not to be homogeneous; at least four different substances could be separated. The properties of "E_4" appeared, therefore, to be the result of the various properties of its components. A slight estradiol contamination was found to be the main cause of its biologic activity.

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THE PRINCIPLES OF IDENTIFICATION AND MEASUREMENT OF VULVAR FLUORESCENCE

RALPH C. BENSON, M.D. AND MARCEL J. VOGEL

The Department of Obstetrics and Gynecology, University of California School of Medicine, and the Research Laboratories of the Vogel Luminescence Corporation, San Francisco, California

COLOR changes in vulvar fluorescence have been correlated with shifting ovarian hormone levels (1, 2). This is an important relationship and it is generally admitted that certain faint color changes do occur from the menarche through the menopause and with pregnancy. There is disagreement, however, as to what hues are actually seen in the perineum and whether true luminescence[1] really is present. In order to establish the clinical reliability of vulvar fluorescence, we have developed the means of determining or excluding definite fluorescence in the vulvar area. We have identified certain luminescence colors and have also established their brightness values in photometric units, but we have not attempted to correlate these changes with endocrine variations at this time. Other colors seen in the pudendum under "black light"[2] have been shown to be of nonfluorescent origin.

All living tissues possess a characteristic fluorescence under ultraviolet illumination (3). Moreover, the degree of fluorescence hue, saturation and brightness varies with health and disease (4, 5, 6). Variations in tissue fluorescence may be expected in bacterial or fungus infections, some degenerative lesions, and certain types of endocrine or metabolic imbalance. Our interest is presently confined to the natural fluorescence exhibited by tissues and body secretions *without* the addition of fluorescent substances. Fluorescence produced by the injection of dyes such as fluorescein is a special phenomenon.

Many aspects of fluorometric medical diagnosis have already been explored with varying degrees of success, yet a means of description of biologic fluorometry is generally lacking. In dermatology, skin fluorescence is an established index for the recognition and differentiation of erythema,

Received for publication December 15, 1954.

[1] The terms "fluorescence" and "luminescence" are synonymous for practical purposes.

[2] The ultraviolet light referred to is filtered, long-wave radiation and is commonly known as "black light" or Wood's light. The filter is a nickel-cobalt glass designed for maximum transmission at 3660°.

the exanthemata, keratoses, xeroderma pigmentosum and certain fungus infections (7–10). Ultraviolet light will delineate interstitial keratitis and outline cataract (11, 12). The fluorescence of scratch wheals indicates the extent and degree of peripheral vascular insufficiency (13). Vitamin B deficiency can be detected by changes in the fluorescence of the tongue (14, 15). Moreover, various surfaces of the body exhibit typical zonal fluorescence, and a rough charting of these phenomena has been attempted (6, 16). Pigmentation of the skin with its vascular alteration has been related to estrogens in the female (17), and to androgens in the male (18).

Changes in vulvar luminescence, from the practical aspect, probably reflect variations and aberrations of female steroid sex hormones, disorders of menstruation, and endocrine hemorrhagic conditions of pregnancy, and serve as an index of the success of treatment (18–21). The determination of vulvar fluorescence has not been widely accepted because of the lack of established standards. The investigator who is interested in this diagnostic approach has had to rely upon his personal observations and experience with the procedure. Hence, a simple classification of biologic variations in luminescence is necessary to pave the way for the use of this method for valid diagnosis and prognosis in female endocrinology and gynecology.

Numerous investigators have attempted the differentiation between benign and malignant lesions by means of specific tissue fluorescence (5, 22–27). Nevertheless, in spite of certain encouraging results in this, as well as in related fields, uncertainty as to the specificity of the visualized phenomena has often thwarted the clinician. The substantial literature on fluorescence in the field of the diagnosis of cancer alone, however, leads to the inescapable conclusion that this procedure has some value. Unfortunately, there has usually been disappointment with the type and quality of the changes observed and with the method for accurately recording these details. This attitude is obvious when one critically re-examines published data. Dissatisfaction has also been expressed by investigators in this field because of the poor photographic color reproductions as compared with the original appearance (5, 28). Photographs of fluorescence in living subjects are unsatisfactory because of body movement during the necessarily prolonged time exposure (29). Therefore, an impersonal, scientific basis for evaluation and description of biologic luminescence is indicated.

Not only do tissues evince individual tones when excited by ultraviolet light, but naturally fluorescing body secretions also have their specific luminescence values. Examples of these are the bluish-white fluorescence of normal urine (30), the red fluorescence of porphyria urine (31–33), and the green fluorescence of urine containing urobilin (34, 35). (We shall not consider the fluorescence of the estrogens in this article because spontaneous luminescence of these substances does not occur. In the

determination of the type and amount of estrone in the urine, for example, a reagent such as sulfuric acid must be added to produce fluorescence (36, 37).

Normal blood in the fluid state is nonfluorescent, although serum (38, 39) and certain blood cells do fluoresce (40, 41). Breakdown products of blood, among them the protoporphyrins and uroporphyrins, emit specific red fluorescence colors (42–45). Perspiration fluoresces a bluish-white (16) or, at times, a curious yellow color (3). Cerumen fluoresces yellow (7). Semen has a strong bluish-white fluorescence (46). A dried deposit of semen is differentiated from a urine stain by its stronger bluish color and its higher fluorometric brightness value (47–49). Feces fluoresce only by virtue of the variable content (50, 51). Superficial ulcerative and neoplastic lesions are known to produce fluorescent porphyrins (52). Leukorrheic discharges fluoresce when these excretions contain porphyrins (53), and this observation has been used as a possible aid in the detection of malignancy, or to suggest cancer susceptibility in gynecologic patients (54).

Normal human and bovine milk exhibits a brilliant yellowish-white fluorescence (55). Although various types of milk display somewhat similar fluorescence, each is distinctive and the types can be differentiated one from the other by fluorometric comparison (56). The nature of the mother's diet and the bacterial contamination of the milk markedly modify the luminescence of this secretion (57, 58). Without the aid of definite criteria for fluorometric comparison, however, it is impossible to express these differentiations in adequate terms.

PROCEDURES AND METHODS IN INVESTIGATION OF BIOLOGIC LUMINESCENCE

Purpose and scope of study

We propose to establish certain standards for differentiating between fluorescing and nonfluorescing surfaces. Moreover, a set of fluorometric color charts for visual comparison of biologic luminescence phenomena will be presented. The dark-adaptation procedure required to eliminate capricious individual interpretations will be outlined. Minimal practical sources of ultraviolet excitation, as well as filter systems, will be appraised and we trust essential requirements for the study of luminescence in clinical medicine may be established.

Fluorescence vs. nonfluorescence

A knowledge of the basic physics and the ability to appraise an object's response to ultraviolet light are necessary for any clinical study involving luminescence. Fluorescence occurs when an object absorbs energy beyond

the visual limits of the human eye, with the re-emission of radiation in the visual spectrum (59). Confusion in fluorometric observations generally results from the simultaneous perception of reflected light passed by the filter, and the actual fluorescence of the object itself (19). While inspecting an object, the observer should move his head from side to side to seek a non-changing color—which will usually be the fluorescence-hue. Lateral viewing is commonly required in order to avoid seeing reflected visible light in vulvar or other surface luminescence (Fig. 1).

Occasionally, the eyes must be protected from ultraviolet radiation, which can excite distracting retinal fluorescence. This can be circumvented

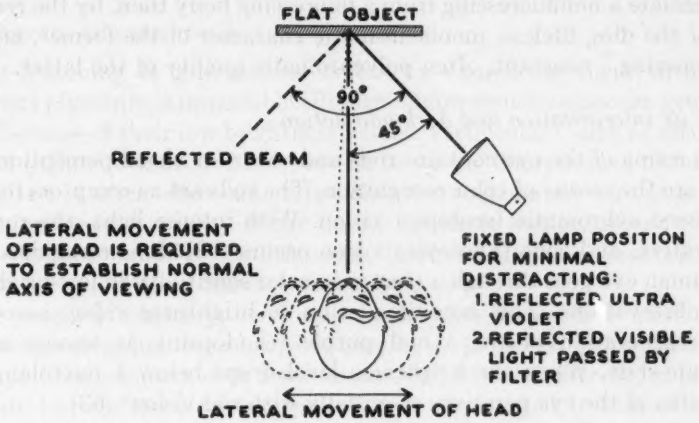

FLAT OBJECT
90°
45°
REFLECTED BEAM
LATERAL MOVEMENT OF HEAD IS REQUIRED TO ESTABLISH NORMAL AXIS OF VIEWING
FIXED LAMP POSITION FOR MINIMAL DISTRACTING:
1. REFLECTED ULTRA VIOLET
2. REFLECTED VISIBLE LIGHT PASSED BY FILTER
LATERAL MOVEMENT OF HEAD

FIGURE 1

by placing ultraviolet-absorbing film before the eyes or wearing goggles (18), but such devices are rarely necessary.

The visually observable phenomena in biologic fluorescence are complex mixtures of colors. Unfortunately, the intensities of most of these colors are so weak that they are at the lower limit of color vision. Hence, there is wide variance in individual interpretation of the degree of luminescence.

Visual spectromety

As a further aid in the defining of certain fluorescing areas, one may employ Wratten photographic filters (red, green and blue)[3] (60). For example, a filter which transmits a narrow band of red light will allow the observation of a fluorescence emission lying within that particular zone. Two apparently identical red colors, which presumably fluoresce similarly,

[3] Kodak Wratten Red A #25, Green B #58, and Blue C5 #47.

can be sharply differentiated by observation through an appropriate filter. Specifically, if one of the two fluorescing objects contains green, viewing through a green filter will disclose this difference. The proportions of red, green and blue radiation present in this area can then be estimated. The fluorescence emission is thus characterized in terms of its components. It is, in effect, a good preliminary means of visual spectrometry.

A fluorescing body has the ability to emit visible light of various hues as an inherent property, whereas a nonfluorescent one can only reflect, refract or absorb visible light. Most ultraviolet filters, imperfect as they are, pass a small percentage of visible red and blue light (61). This causes nonfluorescent objects to appear in dull values of violet or purple. One can differentiate a nonfluorescing from a fluorescing body then, by the recognition of the dim, lifeless, monochromatic character of the former, and the "shimmering," constant, often polychromatic quality of the latter.

Color; its interpretation and dark-adaptation

The retina of the eye contains rods and cones for visual perception. The cones are the means of color recognition. The rods act as receptors for low-brightness achromatic (scotopic) vision. With intense light, the rods are inoperative, and cone (photopic) vision occurs (62). It is recognized that the human eye goes through a change in color sensitivity in low-brightness level observations. This accommodation to brightness value, associated with regeneration of the visual purple (rhodopsin), is known as the Purkinje-shift. When the brightness level drops below 1 microlambert,[4] the retina of the eye perceives essentially with rod vision[5] (63).

There is another zone, the mesopic region, in the retina just peripheral to the fovea centralis, in which there is both rod and cone vision. Here, visual response occurs when there is excitation by radiant energy of from 1 to 200 microlamberts (64), the usual range of vulvar fluorescence study.

If one is working strictly with rod vision, color differentiation is inaccurate. The rods are not a source of color appreciation, but are strictly a means of brightness determination. Furthermore, the accommodation of the eye for rod vision requires from thirty to forty-five minutes in complete darkness. Brightness values above 1 microlambert cause a partial loss of this accommodation (64).

In contrast, the mesopic region of the eye normally takes only from five

[4] One microlambert is equivalent to 1/1,000,000 brightness of a perfectly diffusing, completely reflecting surface illuminated by a source of 1 candlepower at a distance of 1 centimeter.

[5] In comparison, radioactive luminous compounds used on watch dials are from 5–7 microlamberts in brightness.

to seven minutes for dark-adaptation in order to interpret color properly. Our experimental work has repeatedly shown this to be true. The cones also require dark adaptation in order to allow the eye to perceive color (65). No observations in tissue luminescence should be undertaken, therefore, until the viewer has been in near-darkness for at least seven minutes (65).

Valuable data are constantly lost by improper descriptive terminology. For example, hormone therapy often results in minor daily variations in the subject. These changes require precise evaluation and description. Without them, the value of the clinical interpretations is reduced and they become actually deceptive in subsequent elucidation and analysis. This is particularly true in regard to vulvar fluorescence.

Fluorescence color-matching

The matching of fluorescence colors by comparing them with non-luminous pigments is impossible. Biologic fluorescence colors are generally weak because of their low brightness values. Vivid colors, such as saturated hues of yellow or blue, are not present in vulvar fluorescence, although a strong red is often observed. What the viewer generally sees is a faint, tinted luminescent quality of the tissues (intrinsic luminescence). Surface stains, contaminants or medicaments, however, may be responsible for intense color (extrinsic luminescence) (19).

The original arbitrary, obviously inadequate terms first used (1) in describing vulvar luminescence: *deep purple, purple, green, yellow, brown* and *red* have been adopted with reservations (19, 21). Admittedly, it is more accurate to refer the color interpretation of the subject to that of a fixed standard rather than to assign an indefinite color-name to the phenomenon. Just what *is* such a color as *purple, deep purple,* or *green?* We, like others (19, 20), have attempted to answer these questions by trying to match skin fluorescence colors with nonfluorescent crayons. We now know that this cannot be done.

It became obvious to us that a similar *medium* is required for color definition and comparison. Fluorescent tempera colors in a water base proved to be impractical in attempting to match fluorescing areas. Therefore, we developed a series of non-fading easily classified fluorescence color charts to be used as standards for the interpretation of vulvar luminescence.

Each chart was made by a combination of two of the hues listed in Table 1. A basic set of ultraviolet responsive phosphors was chosen as primary fluorescing color standards (Table 2). Each color was assigned a hue number as shown in Table 1. We found that a pure titanium dioxide-based tempera color possessed no fluorescence and established a good index

for a *negative* or zero fluorescence blank. Proportionate blends between two adjacent hues provided progressive color combinations.[6]

Five basic fluorescence color standards are proposed for the description of biologic luminescence. They are: *yellow, orange, red, blue* and *green.*[7] They are specifically defined in Table 2 and substantiate the validity of the values for fluorescence hue and brightness. Figure 2 represents an example of the fluorescence colors from yellow to orange.

TABLE 1. COMPOSITION OF FLUORESCENCE COLOR STANDARDS

Fluorescing color under 3660 A	Our hue No.	Predominant wavelength (μ)	Phosphor-type	New Jersey Zinc Company No.	Brightness value (%)
Yellow	1	586	Zinc cadmium sulfide (manganese)	2266	35
Orange	2	590	Zinc cadmium sulfide (manganese)	2220	35
Red	3	655	Magnesium titanate	2125	2
Blue	4	445	Zinc sulfide (zinc)	2205	25
Green	5	520	Zinc sulfide (copper)	2210	95

The colors to the right of the hue-blends in the color charts are desaturated in proportionate steps. Each color is assigned a definite number which indicates the proportion of the hue-values as well as the saturation. Thus, a color found in the yellow-orange range would be described as Y/O 20.4. The first two digits refer to the proportion of yellow to orange, or 20 per cent yellow and the remainder (80 per cent) orange. The fractional numeral indicates a reduction in brightness-value of 40 per cent.

After preliminary, studies, it was found that only two charts were

[6] These charts, as prepared by Vogel Luminescence Corp., were made by blending carefully ground fluorescent zinc sulfide-type phosphors in a water-color vehicle, which was then brushed onto white-coated paper stock. The reduction in fluorescence-brightness was accomplished by proportionate additions of titanium dioxide tempera-color. All of these standards appear white to near-white in daylight.

[7] A stable and reliable fluorescent purple is yet to be developed. This deficiency in standards is not a problem, however, because adequate matching with the red-blue chart is satisfactory for all practical purposes.

TABLE 2. CHARACTERISTICS OF SMALL COLOR AREAS AGAINST A DARK BACKGROUND (REFERENCE 66)

Color	Color boundary	Average wave-length (μ)	Variation (μ)
Red			
	red or	627	604–656
Orange		598	592–608
	or yellow	589	585–597
Yellow		577	568–585
	yellow-green	566	558–577
Green		512	495–535
	green-blue	495	487–502
Blue		472	452–485
	blue-violet	436	421–456

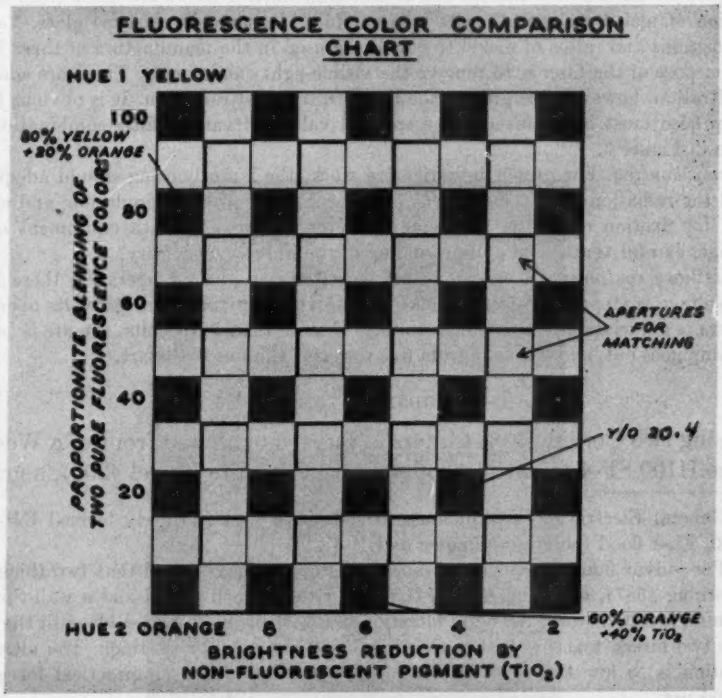


FIGURE 2

actually required in measuring the vulvar luminescence of the parturient woman. The combinations between Hue #3 (red) and Hue #4 (blue), and Hue #4 (blue) and Hue #5 (green), covered the color variations from the antepartum through the postpartum period. The extrapolation of intermediate values from these combinations is simple.

DESCRIPTION OF EQUIPMENT

Sources of ultraviolet light. In the examination of vulvar fluorescence, an intense source of long wave-length ultraviolet radiation is required. The most practical lamp presently available is a high-pressure mercury vapor burner enclosed in a sealed-beam reflector housing. This light source may be obtained in either a spot, or floodlight pattern.[8] The lamp draws 100 watts. An auxiliary transformer is required to operate the lamp unit. The more intense spot light was found by experimentation to be necessary for vulvar luminescence study. Other types of mercury vapor sources are also available. They include low-pressure fluorescent tubular sources, low-pressure quartz burners, 100-watt mercury sources with built-in filters, and carbon-arc lamps. The latter are unsatisfactory for vulvar luminescence studies. The fluorescent tubular sources have clinical application in dermatology, however, because of the adequate brightness-value of many of the fungi (10).

Filters for ultraviolet radiation. Ultraviolet transmitting filters are made from a combination of nickel and cobalt salts in a carefully prepared Pyrex-type glass. Varying compositions and ratios of nickel to cobalt are used in the manufacture of these filters. The purpose of the filter is to remove the visible-light components. The more complete the filtration, however, the greater the loss of ultraviolet radiation. It is obvious that a compromise must be achieved.[9] The spectral values of various filter combinations are shown in Figure 3.

Lamp housing. For proper investigative work, the lamp housing should adequately shield the radiation from the observer (Fig. 4). A handle for manipulation, and often a stand for fixation of the unit, are requirements. In working with equipment of this wattage, careful ventilation and grounding of the unit are necessary.

Auxiliary equipment. A special transformer[10] is required for operating these lights. The voltage is stepped up from 110 AC to 245 volts starting, and 130 volts operating. If there is a variation in the line of voltage of more than 7–10 volts, the arc is broken, the lamp goes out, and will take from five to seven minutes to restart.

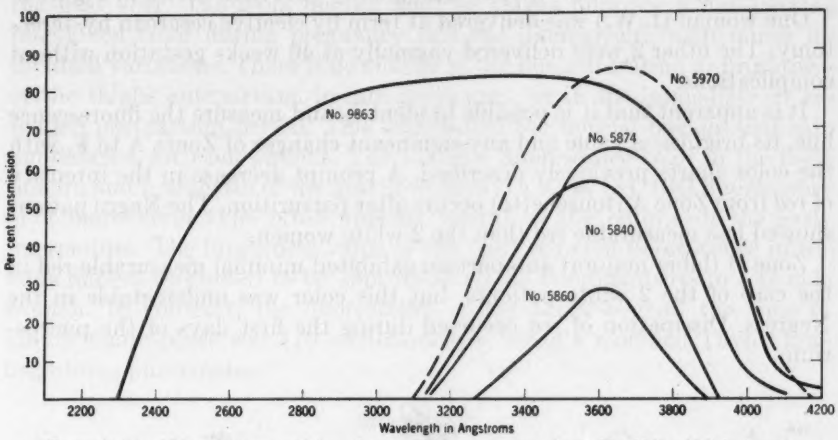
OBSERVATIONS

Using two Corning #5874 filters in series mounted in front of a Westinghouse H100 SP-4 ultraviolet light source (Vogelite Model #203), a graphic

[8] General Electric or Westinghouse H-100, SP-4 spot (formerly termed CH-4); or H-100, FL-4 flood (heretofore known as EH-4).

[9] For vulvar fluorescence study, most investigators have found that two thicknesses of Corning #5874, or of the Kopp #41 filter, with a 5-inch rondel and a wall-thickness of 5 mm. is satisfactory. Absolute filtration of all visible light is possible with the use of these two filters, together with a Corning #5860 filter, 4.95 mm. thick. The ultraviolet radiation is so low through combination, however, that it is impractical for general clinical use.

[10] General Electric 89G 140.



Transmission of ultraviolet transmitting glasses. Thickness 5 mm. except No. 9863 which is 3 mm. *(Corning Glass Co. Catalog.)*

FIGURE 3

FIGURE 4

representation of the vulvar fluorescence color changes in 2 normal light-complexioned obstetrical patients (L.W. and D.A.), and 1 very dark-skinned Negro with uncomplicated pregnancy (F.T.), is shown in Figure 5.

One woman (L.W.) was delivered at term by elective cesarean hysterectomy. The other 2 were delivered vaginally at 40 weeks gestation without complications.

It is apparent that it is possible to identify and measure the fluorescence hue, its brightness value and any significant changes of Zones A to F, with the color charts previously described. A prompt decrease in the intensity of *red* from Zone A (fourchette) occurs after parturition. The Negro patient showed less measurable *red* than the 2 white women.

Zone B (labia majora) antepartum exhibited minimal measurable red in the case of the 2 white patients, but this color was undetectable in the Negress. Dissipation of *red* occurred during the first days of the puerperium.

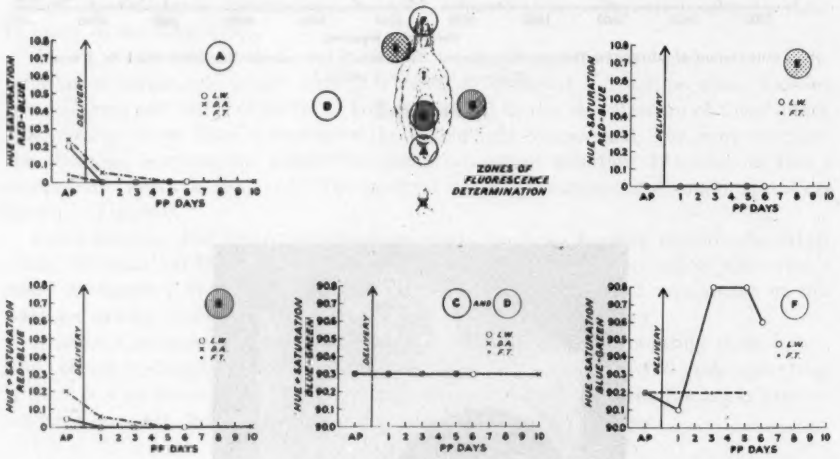


FIGURE 5

A presistent blue-green fluorescence of Zone C (vagina) and Zone D (inner aspect of the thigh) was noted in all 3 patients, without change, before or after delivery.

No *red* was noted at Zone E (area lateral to the clitoris) in the 2 subjects in whom it was studied.

Zone F (prepuce) manifested a variable response. In the colored patient it showed slight, but essentially unvarying blue-green fluorescence during the antepartum and postpartum periods, whereas in the one white patient in whom it was studied, there was an increase in brightness value several days after delivery.

The following observations represent the essence of experience accumulated from extensive preliminary examination of many patients during the past year. The labia majora and the thighs fluoresce a non-varying blue-green color during pregnancy and the puerperium, with minor individual variations. There is no change in the hue, saturation or brightness of the thighs antepartum, in late pregnancy, or in the immediate or extended postpartum period. This established the normal background skin fluorescence for each patient. The variable fluorescence color in the labia minora and fourchette of the gravid woman is a weak red. Red fluorescence of a more vivid type often appears lateral to the clitoris in the early puerperium. The brightness value of the faint red skin fluorescence in the labia minora was noted to be approximately 53 microlamberts in a blonde woman. In contrast, the fluorescence of her thigh beyond the zone of vulvar fluorescence was 170 microlamberts, using a modified Taylor low-brightness photometer.

DISCUSSION

In addition to the major factors of hue, saturation and brightness, there are other variables which modify an appreciation of color. Some of these become very important when low-luminosity and faint colors are considered, as in vulvar fluorescence. These are: size of area, its contour, texture and transparency, and the color of neighboring areas (67).

The fact that most filters pass an appreciable amount of visible red and blue light must be re-emphasized. MacDonald and Margolese (2) stressed the blue visual sensation but did not indicate the significant amount of red that is present. We have proved that there is a gross escape of both these colors by observing the *purple* and *deep purple* skin through a hand spectroscope. Distinct red and blue bands were apparent with two Corning #5872 filters. With an "absolute" filter, the red and blue bands disappeared. A *deep purple* color was apparent with the original filtration, but was absent when additional filtration was employed. We attempted to disregard this "leakage" earlier in our studies (21), preferring to stress the gross differences in pudendal color under ultraviolet light. When precision and actual measurement of the colors were attempted, however, filter deficiencies became a real problem, and special, or more exact filtration of the ultraviolet light was required.

No positive correlation between the advent and degree of skin pigmentation or change in skin texture and the appearance of *purple* or *deep purple* will be drawn in this report. In pregnancy, a definite increase in pigmentation of the pudendum and labia occurs. This varies with the woman's complexion. Slight edema of the labia in late pregnancy and in the early puerperium is common. After delivery, however, there is a reduction in vulvar fullness and obvious wrinkling of the skin occurs, particularly at

the fourchette and in the labia minora. The basic skin color previously called *purple* or *brown* is, in our present opinion, actually blue-green. This hue is modified by *red*, which is the only true variable fluorescence color seen in the perineal area with proper filtrations. The cycle of fading for the brown pigmentation of pregnancy is slow, a matter of weeks, whereas fading of red tissue-fluorescence is complete in a matter of days. We have found wide, and often unexplained, individual variations in the time of appearance and the extent of the red fluorescence. It is particularly difficult to type this phenomenon in colored patients.

It would seem, therefore, that an explanation of the previously described colors is in order. We believe that the *purple* and *deep purple* can be explained on the basis of blue-green fluorescence of the skin, plus filter leakage of red and blue. Darker hues may be due to melanosis, particularly during pregnancy. The *deep purple* of pregnancy may be accounted for by the foregoing plus red skin-fluorescence. In a previous article (21), we mentioned the intensification of the *deep purple*, which is made possible by stretching of the skin at the fourchette. This maneuver removes wrinkles, increases reflection of visible light from the source and, as a result, augments the purple sensation. The presence or absence of definite red fluorescence of the skin is significant in this determination.

Morton's vigorous attempts (68) to modify skin circulation by heat and cold were not productive of appreciable color change. We have noted an increase in blueness of the striae and the appearance of varices during pregnancy and, although this may be reflectance phenomenon, actual increased vascularity must provide some addition to the blue component of the color observed.

Yellow, *green* and *brown* are more difficult to explain. Intrinsic *red* fluorescence is probably minimal or absent in zones in which *yellow*, *green* or *brown* are described. The skin is pigmented and, being essentially brown, it will absorb blue and reflect red or violet. A greenish or yellowish color of the skin is made possible by a neutralization of the blue fluorescing component in the absence of red. A combination of red, blue and green fluorescence produces a whitish luminescence, which also can be confused with pale green or yellow because it is lighter than near-by areas.

CONCLUSIONS AND SUMMARY

1. An extensive appraisal of the medical literature indicates a wide interest in the biologic fluorescence phenomenon but with no adequate means of description.

2. Explicit comparison with fluorescence standards is required to establish the clinical specificity and reliability of vulvar and other biologic luminescence.

3. The visual differences between fluorescent and nonfluorescent surfaces have been established by means of critical viewing and adequate filtration of the ultraviolet light source.

4. Visual spectrometry, using red, green and blue Wratten filters, allows the differentiation of major and minor fluorescence color sensations.

5. A minimum time allowance of seven minutes for dark adaptation has been established for reliable color discrimination in the low-brightness range of color vision.

6. A series of fluorescence color comparison charts, laminated in plastic, was made for clinical use in appraisal of vulvar luminescence. Each graduated unit was prepared with light-stable phosphors of known hue, saturation and brightness.

7. A practical terminology was developed to describe the color-match with the use of these fluorescence standards.

8. An intense, focused light source of long-wave ultraviolet radiation (maximum wave length, 3660 A), together with proper filtration of the visible components, is required for clinical vulvar luminescence studies.

9. The vulvar colors previously described as *purple* and *deep purple* were shown to be nonfluorescence combinations resulting from the blending of visible red and blue passed by the filter, together with the brown pigmentation of the pregnant woman's skin.

10. The normal, constant background fluorescence of the skin and vagina of the pregnant and puerperal woman is not purple but a faint blue-green. Weak to intense *red* is the only variable fluorescence color in the vulva of the antepartum and postpartum woman.

11. A simplified standardized technique and a satisfactory means of description provide a much-needed impersonal scientific basis for evaluation of vulvar and other biologic luminescence.

12. The future status of vulvar fluoresence as a diagnostic index would seem to depend upon the correlation of variations in red luminescence with significant clinical and hormonal variations.

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ENDOCRINE STUDIES IN 8 PATIENTS WITH DYSTROPHIA MYOTONICA

WYMAN E. JACOBSON, M.D.,* ALVIN L. SCHULTZ, M.D.† AND JACK ANDERSON, M.D.‡

The Department of Medicine, St. Louis Park Medical Center, Veterans Administration Hospital, and University of Minnesota Medical School, Minneapolis, Minnesota

DYSTROPHIA myotonica (also called myotonia atrophica) is a relatively rare disease of unknown etiology. In addition to the myotonia the disease is often characterized clinically by cataracts, baldness, hypometabolism and testicular atrophy. It is therefore understandable that various endocrine factors should have been implicated as being causally important. However, objective evaluation of these factors has been incomplete. In the past five years, 8 patients with dystrophia myotonica have been studied in a special effort to evaluate the endocrinologic aspects of this disease. An attempt has been made to study the functions of the thyroid, parathyroid, adrenal, gonadal and pituitary glands.

METHODS

The basal metabolic rate was determined in the accepted manner, using a standard type Benedict-Roth closed circuit respiration apparatus. In patients having several basal metabolism tests, the lowest value was accepted as the most valid. The total blood cholesterol concentration was determined by the method of Schoenheimer and Sperry (1). The thyroidal uptake of radioactive iodine was measured by methods described by Schultz *et al.* (2). The uptake is expressed as the percentage of the tracer dose collected by the thyroid gland in twenty-four hours; in euthyroid subjects the value ranges from 10 to 50 per cent. The serum protein-bound iodine level was determined according to a modification of the method of Barker, Humphrey and Soley (3), as described by Zieve *et al.* (4). By this method the normal concentration of serum protein-bound iodine ranges from 3 to 8 micrograms per 100 ml. Evaluation of adrenal function included the Robinson-Power-Kepler "water test" (5), the eosinophilic response to intramuscularly injected ACTH (6), the eosinophilic response to a slow intravenous injection of ACTH (7), and the determination of the urinary excretion of 17-hydroxycorticoids using a modification of the method described by Reddy *et al.* (8). In some instances the excretion of 17-hydroxycorticoids was also measured after adrenal stimulation by intravenous ACTH. The normal urinary excretion of 17-hydroxycorticoids by this method varies from 3 to 12 mg. per twenty-four hours. The 24-hour urinary excretion of 17-ketosteroids was determined by the method of Klendshoj *et al.* (9). Bioassay of urinary

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* Clinical Assistant Professor in Medicine, University of Minnesota.

† Assistant Professor in Medicine, University of Minnesota.

‡ Resident, Internal Medicine, Veterans Administration Hospital.

gonadotropins was carried out by a modification of the method described by McCullagh and Bowman (10). Normal values, in our experience, range from 12 to 96 mouse units per twenty-four hours. The urinary excretion of creatine was determined by the method of Bonsnes and Taussky (11). In normal adult males there is a negligible excretion of creatine in the urine; values above 100 mg. per twenty-four hours are most likely abnormal.

CASE REPORTS

Case 1

T.K., a 38-year-old male, first noted weakness of his hands in 1942 while in the military service. In 1943 the diagnosis of myotonia was made. In 1948 he first noted the presence of a thyroid nodule. By 1949 he had become impotent, but there was no testicular atrophy. At approximately the same time he was first found to have marked hypometabolism and was given a trial course of desiccated thyroid, without apparent subjective or objective improvement. By 1950 there had been an increase in the size of the thyroid nodule and the patient began having mild pressure symptoms. The nodule was removed and histologic study showed that it was a benign colloid adenoma.

The family history revealed no other cases of myotonia.

Physical examination: The patient was a gaunt white male in no acute distress. His speech was slurred, and of a nasal quality. The head showed nothing abnormal, except for prominent atrophy of the facial muscles. There was a well healed thyroidectomy scar. The eyes were normal and there were no cataracts. Neurologic examination revealed bilateral ptosis and weakness of the orbicularis oculi, weakness and atrophy of the sternocleidomastoids, weakness of the grip bilaterally, paresis of the muscles of the lower extremities below the knees, and a typical myotonic response on percussion of the muscles of the tongue and the thenar eminence.

Laboratory studies: The hemoglobin, the white blood cell count, the erythrocyte sedimentation rate and routine urinalysis findings were consistently normal. Results of a glucose tolerance test were normal, as were the levels of blood urea nitrogen, plasma proteins, and serum chloride. The urinary excretion of creatine was 400 mg. in twenty-four hours. All other laboratory studies are summarized in Table 1.

Roentgenograms showed the chest, skull, long bones, upper gastro-intestinal tract and colon to be normal.

Case 2

G.B., a 38-year-old male, was admitted to the hospital in October 1951, at which time he complained of marked weakness and inability to walk or stand normally. He first noted weakness in his hands and legs in 1944. This had increased gradually over the seven-year period and there had been progressive muscular atrophy, especially in both legs. The diagnosis of myotonia was first made in 1950.

Physical examination: The patient was a thin white male, who appeared chronically ill. He walked with great difficulty and his gait was slapping or "steppage" in type. The head was normal, except for baldness. The eyes were normal and there were no cataracts. The thyroid gland was not enlarged. There was bilateral testicular atrophy. Neurologic examination revealed weakness of the sternocleidomastoid muscles, the flexors of the digits, and of the gastrocnemius and soleus muscles, weak or absent deep tendon reflexes, the presence of Romberg's sign and a typical myotonic reaction on percussion of the thenar eminence and over the biceps muscles.

Laboratory studies: The hemoglobin, erythrocyte sedimentation rate and routine

urinalysis findings were normal. The levels of serum calcium, serum inorganic phosphorus and plasma proteins were normal. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 120 mg. per twenty-four hours. Roentgenograms showed the chest, skull, and long bones to be normal. Testicular biopsy showed marked tubular degeneration and atrophy with normal-appearing Leydig cells (Fig. 1). Muscle biopsy showed typical atrophy and fibrous tissue replacement (Fig. 2).

Case 3

C.H., a 61-year-old male, was admitted to the hospital in September 1953 with primary complaints of weakness, decreased visual acuity, anorexia and slight weight loss. The patient dated the onset of his muscular weakness to 1948, at which time he

TABLE 1. STUDIES OF ENDOCRINE FUNCTION IN 8 PATIENTS WITH DYSTROPHIA MYOTONICA

Patient	BMR (%)	Serum cholesterol (mg./100 ml.)	Thyroidal I^{131} uptake (%)	Serum PBI (γ/100 ml.)	Eosinophil response to ACTH (% fall)		Urine 17-KS (mg./24 hrs.)	Urine 17-OHCS (mg./24 hrs.)	Urine FSH (m.u./24 hrs.)	Serum Ca (mg./100 ml.)	Serum P (mg./100 ml.)	Fasting blood glucose (mg./100 ml.)	Urine creatine (mg./24 hrs.)
					i.m.	i.v.							
T.K.	−34	189	38							9.6		61	400
G.B.	−34	125	27		62		11.9		48–96	9.5	3.7		120
C.H.	−18	130	18	4.7						9.5	4.0	79	320
A.L.	−22	202	15	4.3	70		5.1					256*	220
R.F.	− 8	209	18	4.1	15	87	10.1	1.4†	4–8			254*	125
J.K.	−21	155	36	5.2	14		7.5	4.5		10.2	3.5	82	33
D.M.	−19	200	18	5.2	7	82	8.3	10.0	24–48	10.6	3.7	95*	173
H.S.	−25	170	26	4.5	31	47	11.3	3.4‡	12–24	10.4	3.7	86	625
Mean	*−23.6*	*172*	*24.5*	*4.7*			*9.0*						
SD	*± 8.6*	*±33.0*	*±8.8*	*±0.5*			*±2.6*						

* Oral glucose tolerance curve, diabetic in type.

† After 8-hour infusion of 20 mg. of ACTH, urine 17-hydroxycorticoids rose to 8.2 mg./24 hrs.

‡ After 8-hour infusion of 20 mg. of ACTH, urine 17-hydroxycorticoids rose to 10.9 mg./24 hrs.

noticed difficulty with his gait and weakness of his hands. The patient had noted the development of progressive baldness over the previous five to six years.

The family history was significant in that the patient's 32-year-old son had been discharged from the military service with a diagnosis of "myotonia."

Physical examination: The patient was an elderly, tall, gaunt, white male. There was considerable atrophy of the sternocleidomastoid muscles. Examination of the eyes revealed bilateral cataracts. There was slight testicular atrophy, more prominent on the right. Examination of the extremities showed generalized muscular wasting. A typical myotonic response on percussion of the thenar eminence was elicited and there was marked weakness of the grip bilaterally.

Laboratory studies: The hemoglobin level was 10.7 grams per cent. The total leukocyte count and differential were normal. The erythrocyte sedimentation rate was markedly elevated to 114 mm. per hour. The total serum protein level was elevated to 9.1 grams per cent, with albumin 3.4 grams and globulin 5.7 grams. The value for serum alkaline phosphatase was 7 King-Armstrong units. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 320 mg. per twenty-four hours.

The clinical findings were diagnostic of dystrophia myotonica. Detailed skeletal roentgenograms revealed some osteoporosis of the spine and a few punched-out areas in the frontal portion of the skull. These findings plus the altered concentrations of serum proteins and the markedly elevated sedimentation rate were interpreted as consistent with multiple myeloma. However, bone-marrow aspiration findings were not diagnostic.

Case 4

A.L. was a man in whom the initial diagnosis of dystrophia myotonica was made in 1947. At 57 years of age the patient first noted progressive visual loss. At that time he was found to have bilateral cataracts, and a left cataract operation was performed. At the same time he was admitted to the neurologic service complaining of weakness of his legs dating back to 1938. He was noted to have general muscular weakness and testicular atrophy. He was most recently admitted to the hospital for re-examination in March 1953.

Family history revealed that in all of the patient's 3 sons, signs of dystrophia myotonica had developed.

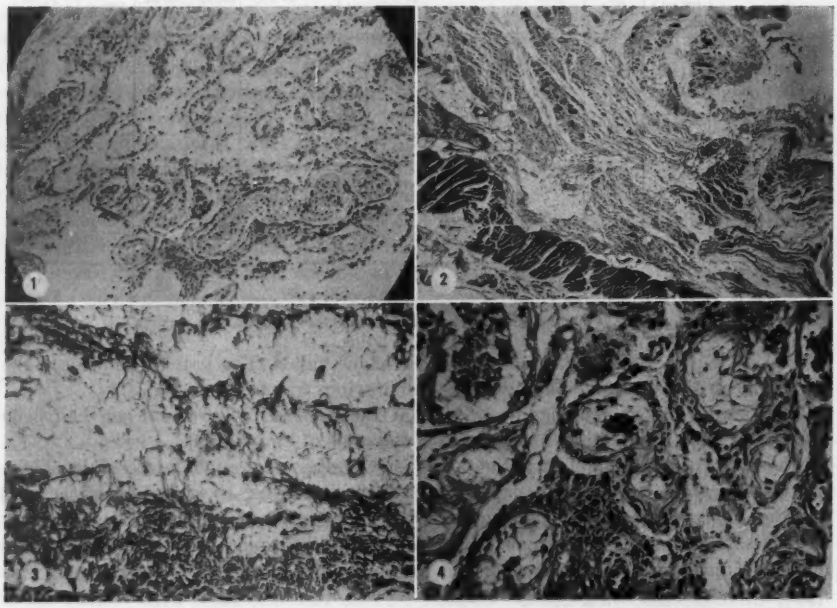

FIG. 1. Testicular biopsy (Case 2, G. B.). Marked tubular degeneration and atrophy but normal-appearing Leydig cells.

FIG. 2. Muscle biopsy (Case 2, G. B.). Typical atrophy of muscle, with fibrous tissue replacement.

FIG. 3. Breast biopsy (Case 7, D. M.). No evidence of glandular structure.

FIG. 4. Testicular biopsy (Case 7. D. M.). Tubular degeneration but normal appearing Leydig cells.

Physical examination: The patient was a white male with typical gaunt facies. There was enlargement of the thyroid gland, with a nodule measuring approximately 1 cm. in diameter. There was bilateral testicular atrophy and considerable muscular wasting.

Laboratory studies: The hemoglobin level was 15.9 grams per cent. The total leukocyte count and differential were normal. The erythrocyte sedimentation rate was 12 mm. per hour. The urine showed a 4 plus reaction for sugar. A glucose tolerance curve was diabetic in type. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 220 mg. per twenty-four hours.

Case 5

R.F., a 31-year-old white male, first showed evidence of dystrophia myotonica during hospitalization in January 1954. He had been known to have diabetes mellitus since 1941 and had been admitted to this hospital on three previous occasions for complications of diabetes. Five years prior to the last admission he had noted a mass in the neck. This had gradually increased in size and had caused dysphagia for about one year. His other complaints included decreased vision and weakness of his hands.

Family history revealed that 1 grandparent had diabetes. His father and 1 sister probably had dystrophia myotonica.

Physical examination: There was considerable atrophy of the sternocleidomastoid muscles. Weakness was noted in both upper and lower extremities. There was a typical myotonic response in the hands. Examination of the eyes revealed immature cataracts bilaterally and evidence of early diabetic retinopathy. In the neck there was a 5-centimeter nodule in the right lobe of the thyroid. Both testes were soft and atrophic.

Laboratory studies: The hemoglobin level, leukocyte count and erythrocyte sedimentation rate were normal. Routine urinalyses were not remarkable except for glycosuria. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 125 mg. per twenty-four hours.

Case 6

J.K., a 28-year-old male, was admitted to the hospital in April 1954. About four months prior to admission he became aware of weakness, muscular pains and inability to "let go" after grasping objects with his hand. There had been progressive loss of libido for about two years.

Family history revealed that previously 1 sister and 1 brother had a condition diagnosed as dystrophia myotonica.

Physical examination: The patient was a thin male with frontal baldness and a gaunt face. The eyes were normal and there were no cataracts. Both testes were atrophic. There was generalized weakness and pronounced atrophy of the sternocleidomastoids, the interossei, the dorsiflexors of the feet and the quadriceps. There was a typical myotonic response on percussion of the thenar eminence.

Laboratory studies: The hemoglobin level and leukocyte count were essentially normal. The erythrocyte sedimentation rate was 33 mm. per hour. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 33 mg. in twenty-four hours.

Case 7

D.M., a 32-year-old male, was admitted to the hospital in March 1954. The patient had previously been studied at this hospital in 1948, at which time he was found to have gynecomastia and hypogonadism. A diagnosis of primary seminiferous tubular failure

and psychoneurosis was made at that time. On admission in 1954 he complained of weakness and difficulty with locomotion, which had been present for many years. He had also noted progressive baldness for fifteen years.

Family history revealed that the patient's father in all probability had dystrophia myotonica.

Physical examination: The patient was a well developed male with marked baldness. There were no cataracts. The thyroid gland was not palpably enlarged. There was evidence of pseudo-gynecomastia, and marked testicular atrophy. Neurologic examination revealed atrophy of the forearms, weakness of the sternocleidomastoid muscles bilaterally, a bilateral foot drop, weakness of the dorsiflexors of the feet and a typical myotonic response on percussion of the thenar eminence.

Laboratory studies: The hemoglobin level was 14.6 grams per cent, and the total leukocyte count was 6,700, with a normal differential. The erythrocyte sedimentation rate was 7 mm. per hour. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 173 mg. in twenty-four hours. A glucose tolerance curve was diabetic in type. Biopsy of breast tissue failed to reveal any evidence of glandular structure and showed only the presence of fat tissue (Fig. 3). Testicular biopsy showed marked tubular degeneration and atrophy (Fig. 4).

Case 8

H.S., a 29-year-old male, was admitted to the hospital in May 1954 complaining of weakness and loss of functions of his hands. His symptoms had been present for three to four years but had become progressively severe only during the previous two months.

Family history failed to disclose any definite cases of dystrophia myotonica in other members of the family.

Physical examination: The patient was a tall thin male, with marked baldness. There was no evidence of cataracts. The thyroid gland was not enlarged, and the testes were not atrophic. Neurologic examination revealed considerable atrophy and weakness of the sternocleidomastoid muscles. There was slight atrophy of the forearm muscles and a typical myotonic response was noted on percussion of the thenar eminence.

Laboratory studies: The hemoglobin level was 13.9 grams per cent. The total leukocyte count was 7,800, with a normal differential. The erythrocyte sedimentation rate was 47 mm. per hour. All special laboratory studies are summarized in Table 1. The urinary excretion of creatine was 625 mg. per twenty-four hours. A glucose tolerance curve was normal.

RESULTS

The clinical characteristics of dystrophia myotonica were well demonstrated by the 8 patients in this study. Three had cataracts, 3 had nodular goiters, 6 had testicular atrophy, and 7 had variable degrees of baldness. All patients had normal skeletal development and normal secondary sexual characteristics.

Seven patients had basal metabolic rates lower than -17 per cent and the mean value for the entire group was -23 per cent (± 9). The serum cholesterol level was uniformly normal and the mean value was 172 mg. per 100 ml. (± 33.0). The thyroidal uptake of radioactive iodine was also uniformly normal, with a mean value of 24.5 per cent (± 8.8) in twenty-four hours, which was comparable to the findings in a previously studied

control group. Chemical determination of the serum protein-bound iodine concentration was performed in 6 of the patients and the results were consistently normal, the mean value being 4.7 gamma per 100 ml. ($\pm$0.5).

In 4 patients there was a subnormal decrease in the circulating eosinophils after the intramuscular injection of ACTH, and in 2 patients there was a reduced excretion of 17-hydroxycorticoids in the urine. However, in each instance the slow intravenous injection of a single dose of ACTH induced either a normal reduction in the circulating eosinophils or a normal increase in the urinary excretion of 17-hydroxycorticoids. Results of a Kepler-Power-Robinson "water test" were normal in the 3 patients tested (T.K., G.B. and A.L.) and insulin tolerance was normal in 1 patient (T.K.).

The mean value for the urinary excretion of 17-ketosteroids as determined in 6 patients was 9.0 mg. per day ($\pm$2.6), which is significantly less than the mean value in normal adult males as reported by Tompsett (12). However, Howard *et al.* (13) consider the range for normal males to lie between 8 and 18 mg. per day.

The urinary excretion of gonadotropin was determined in only 4 patients; it was low in 1, normal in 2, and "high-normal" in 1.

The serum calcium and serum inorganic phosphorus levels were consistently normal. The fasting blood sugar concentration was determined one or more times in 7 of the patients. Two patients had high fasting values and diabetes mellitus of sufficient severity to require strict dietary control and insulin. One patient had a borderline fasting blood glucose level, but a definitely diabetic type of glucose tolerance curve. The glucose tolerance curve was normal in 2 of the remaining patients.

The urinary excretion of creatine was studied in all patients and values in excess of 100 mg. per twenty-four hours were noted in 7 patients.

DISCUSSION

A low basal metabolic rate is an almost constant finding in dystrophia myotonica (14). It was noted previously by Ravin and Waring (15) that clinical myxedema is not present and the serum cholesterol level is usually normal. Our studies would seem to add convincing proof that thyroid function is normal in this disease and that the hypometabolism is extrathyroidal. Atrophy of muscle and partial fibrous-tissue replacement such as occurs in dystrophia myotonica would reduce the total oxygen requirement of the body without a comparable change in the surface area, and thus could result in a low basal metabolic rate independent of thyroid function.

The testicular histologic picture of tubular degeneration and sclerosis with preservation of normal Leydig cells in dystrophia myotonica has previously been reported by Benda and Bixby (16) and by Howard *et al.*

(13). Excretion of gonadotropin (FSH) has been measured in only a small number of patients with this disease and the results have been somewhat variable. Our studies in 4 of the patients with dystrophia myotonica suggest that the testicular atrophy is associated most commonly with a normal urinary excretion of gonadotropin. A reduced urinary excretion of 17-ketosteroids would appear to be a frequent finding in this disease. In our study the urinary excretion of 17-ketosteroids was frequently in the so-called "normal range," though the mean value was distinctly reduced. There was no apparent correlation between the level of the urinary 17-ketosteroid and the clinical degree of testicular atrophy. The hypogonadism of dystrophia myotonica is somewhat similar to "sclerosing tubular degeneration" (Klinefelter's syndrome), in which the urinary 17-ketosteroids may be low in spite of "normal" or "hyperplastic" Leydig cells. The similarity, however, is not complete, since in this disease the Leydig cells are not clumped or hyperplastic and there is not a consistent elevation of the urinary gonadotropin excretion. At the present time it would appear difficult to classify the hypogonadism of dystrophia myotonica, using either the method of Howard *et al.* (13) or that of Heller and Nelson (17). Using the classification of Albert *et al.* (18), the hypogonadism associated with this disease would probably fall into their group II, namely, "Testicular disease in which mature Leydig cells are present," and would be further classified into the subgroup in which the Leydig cells are normal and the major gonadal problem is sterility. We would conclude that in dystrophia myotonica there may occur germinal tissue degeneration with reduction in testicular size and sterility, without any profound hormonal imbalances such as abnormal skeletal development or loss of secondary sexual characteristics.

Of considerable interest in this study was the detection of diabetes mellitus in 3 of the 8 patients. Stanbury, Goldsmith and Gillis (19) recently reported 2 patients with dystrophia myotonica; 1 also had diabetes mellitus. In the report of Caughey and Brown (20), 2 patients were described as having a "decreased glucose tolerance." We are unaware of any other studies reporting an increased incidence of diabetes mellitus in patients with dystrophia mytonica. However, it would appear that there may well be a true increase in the incidence of abnormal carbohydrate metabolism in dystrophia myotonica.

SUMMARY

1. An attempt has been made to evaluate endocrine functions critically in 8 patients with dystrophia myotonica. There was no evidence from this study that this is a disease of endocrine etiology. The data showed, never-

theless, that the basic pathologic process of tissue atrophy may involve certain glandular structures.

2. The basal metabolic rate was often markedly reduced and the mean value for the group was −23 per cent (±9). However the serum cholesterol and protein-bound iodine levels, and the thyroidal uptake of radioative iodine were consistently normal. The hypometabolism of this disease is therefore extrathyroidal in origin and is most likely related to the loss of active muscle tissue.

3. The response of the adrenal cortex and the function of the parathyroid glands were normal.

4. Testicular atrophy was common and the histologic picture was one of tubular atrophy and degeneration with preservation of normal-appearing Leydig cells. The urinary excretion of 17-ketosteroids was often reduced but the urinary excretion of gonadotropin was normal. There were no marked hormonal imbalances attributable to the hypogonadism.

5. Diabetes mellitus was discovered in 3 of the patients. The significance of this observation is unknown.

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THE RESPONSE OF THE I^{131}-TREATED THYROID GLAND TO THYROTROPIC HORMONE*

JORGE M. MARTIN, M.D. AND JOHN B. STANBURY, M.D.

The Department of Medicine of the Harvard Medical School and the Medical Service of the Massachusetts General Hospital, Boston, Massachusetts

THE administration of I^{131} impairs the production of hormone by the thyroid gland. This effect is employed in the treatment of patients with hyperthyroidism (1–4) and in reducing the secretion of thyroid hormone in patients with incapacitating heart disease (5, 6).

Histologic examination of thyroid tissue from patients who received I^{131} therapeutically reveals tissue destruction, fibrosis, cellular hypertrophy, and alteration in the chromatin pattern of the nuclei (7–10). Similar changes have been seen in the rat thyroid after large doses of I^{131} (11). Among the functional derangements demonstrable in the irradiated rat thyroid are impaired responsiveness to iodide deficiency, and to antithyroid drugs, and diminished avidity for I^{131} (11).

Apart from histologic changes and therapeutic response, the effects of I^{131} upon the human thyroid gland have not been thoroughly studied. Kurland, Freedberg and Fishman (12) observed an increase in I^{131} retention and turnover by the I^{131}-irradiated thyroid gland of euthyroid patients when thyrotropic hormone was given. One of the suspected hypothyroid patients reported by Jefferies *et al.* (13) who had received I^{131} for hyperthyroidism, failed to respond to thyrotropic hormone administration. Levy *et al.* (14), also studying the effect of thyrotropic hormone on the I^{131} uptake and the serum protein-bound iodine level in cases of hyperthyroidism after I^{131} therapy, and in normal persons, found that the responses were not always parallel; some patients had an increase in I^{131} uptake without a rise in the serum concentration of protein-bound iodine, and others had a rise in the latter with no change in uptake.

The present communication reports the effect of therapeutic doses of I^{131} on the response of the human thyroid gland to the administration of thyrotropic hormone, as observed in this laboratory.

CLINICAL MATERIAL AND METHODS

The 12 experimental subjects received an unrestricted diet. None used iodized salt. A clinical diagnosis of Graves' disease had been made in all except one, who had a toxic

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nodular goiter. The diagnoses had been established by demonstrating significant elevations of the basal metabolic rate, I^{131} uptake, and serum concentration of protein-bound iodine. Two cases (Nos. 1 and 2) had been treated with I^{131} because of recurrence of thyrotoxicosis after thyroidectomy performed five and two years previously, respectively.

The thyrotoxicosis in 10 of these patients had responded to an initial single dose of I^{131}; in 2 it had required second doses because of persistence. The most recent therapy dose had been given eight months previously. The total dose of I^{131} varied from 8 to 25 millicuries. The percentage of the therapeutic dose retained at the end of forty-eight hours was over 55 per cent, except in 1 patient (No. 12) who retained only 41 per cent. This patient had been receiving potassium iodide for one year, until a month before the I^{131} treatment.

The thyrotoxicosis had been treated with one or two therapeutic doses of I^{131} from eight months to six years prior to the present study. All patients were judged to be clinically euthyroid or only moderately hypothyroid at the time of the study. In 5 the serum concentration of protein-bound iodine was less than 3.5 micrograms per 100 ml.; in 3 of these, the 24-hour thyroidal I^{131} uptake was less than 15 per cent.

The same preparation of thyrotropic hormone[1] was used in all patients. Each dose was injected intramuscularly deep into the buttocks. At the beginning of each experiment the patient was given an oral tracer dose of approximately 5 microcuries of I^{131} and blood was drawn for determination of protein-bound iodine by a modification of the method of Barker (15). The uptake of I^{131} was determined twenty-four hours later in a four-channel scintillation array (16). The patient then received intramuscularly 10 USP units of thyrotropin diluted in 4 ml. of physiologic saline on three consecutive days. One patient (No. 12) received only one intramuscular injection of thyrotropin because of an unusual response (described subsequently). Another patient (No. 8) received only 5 USP units of thyrotropin daily for three days. Immediately after the last injection, he received another tracer dose of 20 microcuries of I^{131}. The uptake was again measured after twenty-four hours, the result being corrected for I^{131} remaining in the neck from the previous tracer. A second sample of blood was drawn for determination of serum protein-bound iodine concentration.

Six other patients, all females, served as controls. The experimental procedure was identical. All were clinically euthyroid and had normal serum concentrations of protein-bound iodine and normal thyroidal I^{131} uptakes. None had previously received I^{131}. Of these 6 patients, 2 had a solitary thyroid nodule, 1 had a multinodular goiter, and the others had diabetes mellitus, rheumatoid arthritis and gastric ulcer, respectively (Table 1).

RESULTS

I^{131}-Treated Patients

The response to administration of thyrotropic hormone appeared to vary with the functional state of the thyroid gland. From Table 1 it may be seen that Patients 1, 2 and 3, who had low initial values for serum protein-

[1] Thyrotropin Armour; Lot No. R491075. Each vial contained approximately 10 USP units of thyrotropin, corresponding to 25 mg. equivalents of the Armour 2R3 standard.

TABLE 1

Patient			Clinical diagnosis	Total dose I^{131} (mc.)	Percentage last dose retained in 48 hrs.	Months since last I^{131} therapy	Thyroidal uptake of I^{131} in 24 hrs. (per cent of dose)*		Serum PBI (μg./100 ml.)		Change*	
No.	Age (yrs.)	Sex					Before TSH	After TSH	Before TSH	After TSH	I^{131} uptake	PBI
1	46	F	Treated Graves'; hypothyroid	6.3	77.9	8	12.1±1.0	15.0±0.5	1.7	1.6	+ 2.9±1.1	− 0.1
2	48	F	Treated Graves'; hypothyroid	10.0	77.4	60	14.7±1.4	21.0±0.8	0.6	1.1	+ 6.3±1.6	+ 0.5
3	28	M	Treated Graves'; euthyroid	25.0	79.0	24	10.0±1.5	23.4±0.6	2.9	2.7	+13.4±1.6	− 0.2
4	45	F	Treated Graves'; euthyroid	10.0	92.0	50	58.3±2.0	57.1±0.8	2.7	3.8	− 1.2±2.2	+ 1.1
5	45	F	Treated Graves'; euthyroid	8.0	89.0	24	36.4±1.2	39.0±0.8	3.3	3.9	+ 2.6±1.7	+ 0.6
6	33	F	Treated Graves'; euthyroid	8.0	69.0	56	28.4±1.7	26.2±1.0	5.1	4.6	− 2.2±2.0	− 0.5
7	52	F	Treated Graves'; euthyroid	9.0	72.0	42	27.0±1.3	22.9±0.8	4.2	4.4	− 4.1±1.5	+ 0.2
8†	48	M	Treated Graves'; euthyroid	9.0	85.0	30	42.5±1.5	45.4±0.8	5.6	4.9	+ 2.9±1.7	− 0.7
9	42	M	Treated Graves'; euthyroid	17.6	59.8	36	17.8±2.2	34.6±0.7	4.8	5.9	+16.8±2.3	+ 1.1
10	38	F	Treated Graves'; euthyroid	9.6	71.0	10	54.8±1.6	85.9±1.2	4.6	10.9	+31.1±2.0	+ 6.3
11	78	F	Toxic nodular goiter; euthyroid	9.0	77.5	18	27.4±1.6	61.0±1.0	6.0	8.2	+33.6±1.9	+ 2.2
12‡	59	M	Treated Graves'; euthyroid	12.0	41.0	72	43.8±1.5	75.5±1.1	7.1	16.7	+31.7±1.9	+12.7
13	25	F	Solitary thyroid nodule	—	—	—	54.3±0.8	87.3±0.9	4.6	6.6	+33.0±1.2	+ 2.0
14	54	F	Diabetes mellitus	—	—	—	36.8±1.2	63.7±0.8	6.7	9.7	+26.9±1.8	+ 3.0
15	77	F	Rheumatoid arthritis	—	—	—	40.3±2.5	69.2±1.2	5.9	10.7	+28.9±2.8	+ 4.8
16	30	F	Solitary thyroid nodule	—	—	—	13.0±2.1	78.7±1.0	4.1	11.2	+65.7±2.3	+ 7.1
17	56	F	Gastric ulcer	—	—	—	23.6±1.4	89.6±0.9	5.8	11.8	+66.0±1.7	+ 6.0
18	50	F	Multinodular goiter	—	—	—	50.0±2.8	63.7±1.1	4.6	12.6	+13.7±3.0	+ 8.0

* Standard deviation due to statistics of counting only.
† Received only 5 USP units of TSH daily.
‡ Received only one injection of 10 USP units of TSH.

bound iodine, failed to manifest a rise in response to thyrotropin.[2] The thyroidal I^{131} uptakes were low initially, and only 1 patient had a slight rise. Patient 4 had a slightly high I^{131} uptake, and Patient 5 a normal uptake, but both had protein-bound iodine concentrations which were below 3.5 micrograms per 100 ml. These 2 patients also failed to respond to thyrotropic hormone.

Patients 6, 7, and 8 had normal control values for serum protein-bound iodine and thyroidal I^{131} uptake. These 3 patients also failed to show a significant response to thyrotropic hormone, either in blood hormonal iodine or in thyroidal I^{131} uptake. In Patient 9 the response was somewhat similar, but there was a moderate increase in I^{131} uptake.

The 3 remaining patients in the I^{131}-treated group responded to thyrotropic hormone. The initial protein-bound iodine concentration and the thyroidal I^{131} uptake were normal in all 3. These patients showed good responses, both in I^{131} uptake and the level of serum protein-bound iodine. One of them (Patient 12) had an unusually vigorous response to thyrotropic hormone: within twenty-four hours after a single injection of 10 USP units there was an enormous enlargement of the thyroid gland. The gland was tender and warm to touch, and there were clinical signs of thyroid overactivity, including transient auricular fibrillation. The serum concentration of protein-bound iodine and the thyroidal uptake of I^{131} increased. Needle biopsy specimens taken at the height of the reaction and while it was subsiding two days later showed no evidence of an inflammatory reaction or interstitial hemorrhage. Five days later the serum concentration of protein-bound iodine was normal. The responses are illustrated in Figures 1 and 2.

There was no evident correlation of the response to thyrotropin with the time that had elapsed since the therapy dose had been given, the percentage of the dose retained, or the age or the sex of the patient.

Control Patients

The data of the control patients are also shown in Table 1. In all instances there was a brisk increase in both thyroidal I^{131} uptake and protein-bound iodine concentration following the administration of thyrotropin. Only 1 of the 6 patients failed to show a rise in protein-bound iodine concentration into the abnormal range, and all showed increases in thyroidal I^{131} uptake into the abnormally high range. In spite of these changes, none of the patients complained of any noteworthy discomfort as a result of the

[2] Changes in serum concentration of protein-bound iodine of less than 1.5 micrograms per 100 ml., and of thyroidal I^{131} uptake of less than 10 per cent, cannot be considered significant. It is possible, however, that changes of physiologic significance can occur which are well within the limits of error of the experimental methods.

procedure, except for occasional slight tenderness in the region of the thyroid.

COMMENT

These data indicate that therapeutic doses of radioactive iodine may impair the response of the thyroid gland to administration of thyrotropic

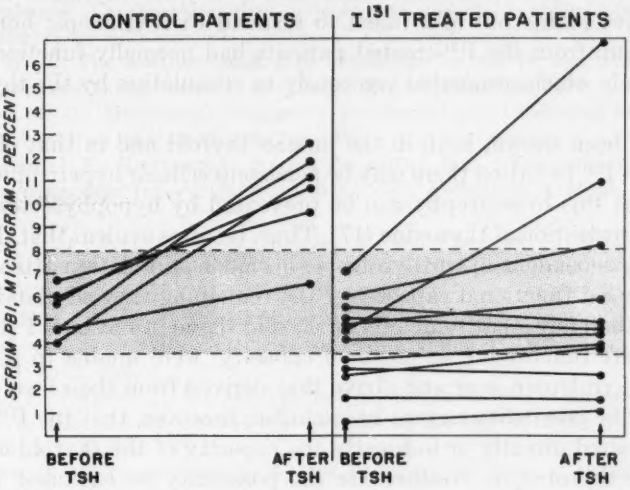

FIG. 1. The response of the serum protein-bound iodine level to administration of thyrotropic hormone in normal subjects (left) and in those previously treated for thyrotoxicosis with I^{131} (right). The normal range of concentration of protein-bound iodine in the serum is shown by the stippled area.

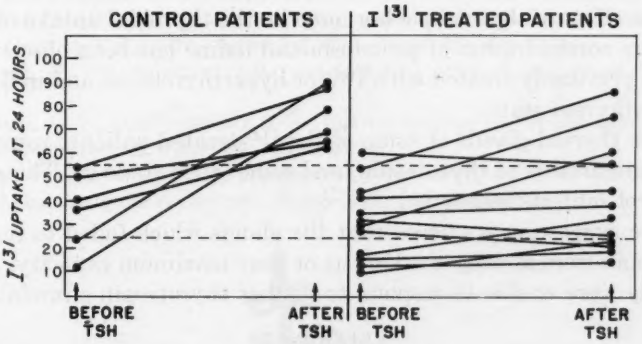

FIG. 2. The response of the 24-hour thyroidal I^{131} uptake to administration of thyrotropic hormone in normal subjects (left) and in those treated with I^{131} (right). The dashed lines indicate the normal limits.

hormone. The patients could be arbitrarily divided into three groups. Some had the functional activity of their thyroid glands so reduced by I^{131} that they failed to maintain a normal serum concentration of protein-bound iodine and a normal thyroidal uptake of radioactive iodine. The glands of these patients showed little or no response to thyrotropic hormone stimulation. Patients in a second group were able to maintain a normal serum concentration of protein-bound iodine and a normal thyroidal uptake of radioactive iodine but also failed to respond to thyrotropic hormone. A third group from the I^{131}-treated patients had normally functioning thyroid glands which responded vigorously to stimulation by the thyrotropic hormone.

It has been shown, both in the human thyroid and in that of the rat, that after I^{131} radiation there may be persistent cellular hypertrophy (9, 11). In the rat this hypertrophy can be prevented by hypophysectomy or by the administration of thyroxine (17). Thus, it seems evident that the hypertrophy is dependent upon thyrotropic-hormone stimulation attendant upon a reduced functional capacity of the remaining thyroid gland. It may well be, therefore, that remnants of thyroid tissue in Patients 1 through 6, which were functioning at maximal capacity, were unable to respond to further thyrotropin over and above that derived from their own pituitary glands. The possibility cannot be excluded, however, that the I^{131} in some way impaired directly or indirectly the capacity of the thyroid cell to respond to thyrotropin. Neither can the possibility be excluded that had thyrotropin been administered in higher dosage or over a prolonged period, there might have been significant stimulation of the thyroids of those I^{131}-treated patients who failed to respond to the dosage which was given.

SUMMARY

1. The effect of thyrotropic hormone on the thyroidal uptake of I^{131} and the serum concentration of protein-bound iodine has been observed in 12 patients previously treated with I^{131} for hyperthyroidism and in 6 subjects in the euthyroid state.

2. The thyroid glands of some of the I^{131}-treated patients responded to the administration of thyrotropin, and some failed to do so. The glands of all control subjects responded.

3. The suggestion is offered that the glands which failed to respond to thyrotropin were already working at or near maximum capacity, and consequently were unable to respond to further thyrotropin stimulation.

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EFFECT OF EPINEPHRINE ON PLASMA LIPID COMPONENTS AND INTERRELATIONSHIPS IN NORMAL AND EPILEPTIC HUMANS

ABRAHAM DURY, PH.D. AND CARELTON R. TREADWELL, PH.D.*

The Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pennsylvania

THE chemical composition of the blood of epileptics has been examined by several investigators for differences from the normal which might indicate the mechanism producing the convulsive state, or further characterize the condition (1). Thus far biochemical studies of this type have been unfruitful. In a different approach to this problem, Dury *et al.* (2) used epinephrine as a "single agent stimulus" affecting many metabolic parameters to determine whether differences could be elicited in specific blood constituents between normal and epileptic subjects. They found that a pattern of changes in the concentration of plasma potassium and glucose appeared after epinephrine administration in epileptics, which differed significantly from responses found in normal subjects.

An acute ketonemia and ketonuria after epinephrine in normal subjects was reported by Hubbard (3). This presumably was referable to the inhibitory action of epinephrine upon the combustion of carbohydrates by peripheral tissues (4). It is of interest in this regard that in the study by Dury *et al.* (2) it was shown that the plasma glucose concentration in epileptics after epinephrine was significantly less at twenty minutes than in comparable normal subjects, and the shape of the blood glucose curve over a period of one hundred and twenty minutes was depressed in the epileptic group compared with the glycemic curve in the normal group. The present study was undertaken as an extension of the latter study, to determine the effect of epinephrine on the concentrations of plasma lipid components and the relationships of these lipid fractions in normal and epileptic individuals.

MATERIAL AND METHODS

Eight normal[1] adult male humans and 10 adult male epileptic patients[2] were the subjects of this study. The ages in the two groups ranged from 21–41 years, and 23–47 years.

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* Guest Investigator, The Dorn Medical Research Laboratory. Permanent address: Professor of Biochemistry, The George Washington University School of Medicine, Washington, D.C.

[1] Members of the laboratory and hospital staffs.

respectively. The nature of the experiment was not known to any of the subjects, except that an intramuscular injection of a "non-harmful" substance would be given and blood samples taken for "tests." All subjects had experienced procedures of this type in the past. The epileptic patients were well-nourished individuals engaged in a variety of indoor or outdoor custodial duties with no other complaints than their epileptic condition.

Blood samples were obtained without stasis from one of the antecubital veins following an overnight fast. Blood was drawn without previous treatment, or one hour after an intramuscular injection of 0.4 mg. of epinephrine (Adrenalin chloride, aq.; Parke, Davis & Co.). The plasma was removed immediately after centrifugation and an aliquot taken for extraction of lipids by a modification of the method of Bloor (5). The combined alcohol-ether extractions were distilled under reduced pressure and the residue taken up in petroleum ether (b.p. 30–60° C.). This was washed three times with an equal volume of water, dried over anhydrous sulfate, and then made to a convenient volume with petroleum ether. Suitable aliquots were taken for the following determinations: total lipids, from weight of residue after distillation and drying to constant weight; lipid phosphorus, by a combination of the methods of Youngsburg and Youngsburg (6) and Fiske and SubbaRow (7); phospholipids, calculated from the lipid-phosphorus values ×26; total and free cholesterol, by the method of Schoenheimer and Sperry (8); and neutral fat content, obtained by difference.

RESULTS

The mean concentrations of the plasma lipid components in normal and epileptic human subjects in the postabsorptive state without previous treatment and sixty minutes after intramuscular administration of epinephrine are shown in Table 1. There were no differences in the concentrations of any of the lipid fractions and the total lipids between the two groups of subjects without previous treatment (Groups A–C). Following epinephrine injection in these same subjects the results showed the following significant differences in plasma lipid components between the two groups: 1) the free, esterified, and total cholesterol concentrations in the epileptic group were significantly greater than the respective values in the normal subjects, and 2) the neutral fat fraction in the treated normal group was significantly greater than in the epileptics (B–D). These results indicate that insofar as the concentrations of the plasma lipid components were concerned, there was a distinction in the response to epinephrine between the normal and the epileptic subjects. The result-statistics presented in columns A–B and C–D provide some indication of the nature of the difference in response to epinephrine between normal and epileptic groups, re-

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[2] Inhabitants of Craig Colony, Sonyea, N. Y., of the State of New York Department of Mental Hygiene. All were identified as idiopathic epileptics through clinical history and/or electroencephalographic records. The only regular medication taken by these subjects was phenobarbital, three times daily.

Grateful acknowledgment is made to Drs. Brill and Bonafee of Craig Colony for their cooperation during the course of this investigation.

TABLE 1. CONCENTRATIONS OF PLASMA LIPID COMPONENTS IN POSTABSORPTIVE NORMAL AND EPILEPTIC INDIVIDUALS, BEFORE, AND SIXTY MINUTES AFTER 0.4 MG. OF EPINEPHRINE INTRAMUSCULARLY

Component (mg./100 ml.)	Normal subjects		Epileptics		"p" values of differences between means in groups:			
	Controls (8)* A	Treated (6)† B	Controls (10) C	Treated (10) D	A-C	A-B	C-D	B-D
Total lipids	636.0‡ ±35.6	705.3 ±46.8	641.5 ±35.6	681.9 ±35.9	ns§	ns	ns	ns
Phospholipids	199.1 ±25.9	203.0 ±25.5	176.2 ± 9.4	231.8 ±11.4	ns	ns	<.01	ns
Total cholesterol	191.7 ±10.1	152.4 ±20.7	208.5 ±12.9	211.8 ±11.8	ns	ns	ns	.025
Free cholesterol	51.0 ± 4.8	43.0 ± 7.2	60.5 ± 3.9	59.8 ± 3.0	ns	ns	ns	.04
Ester cholesterol	140.6 ± 8.1	109.3 ±13.6	148.0 ± 9.1	152.0 ± 8.5	ns	ns	ns	.025
Neutral fat	142.5 ±23.5	270.2 ±31.1	148.7 ±22.3	119.0 ±13.6	ns	<.01	ns	<.01

* Figures in parentheses are numbers of subjects in each group.

† Two of the normal subjects were not available for the epinephrine injection.

‡ Mean ± S.E.

§ Non-significant.

spectively. In the normal group the outstanding effect upon the constitution of plasma lipids after epinephrine administration was the significantly increased concentration of the neutral fat fraction, whereas similar treatment of the epileptic subjects (C–D) induced a significant increase in the concentration of the phospholipid fraction.

The interrelationships of certain plasma lipid constituents in normal and epileptic groups and the effect of epinephrine on these relationships are presented in Table 2. The results show that in the postabsorptive state without treatment the mean ratios of total cholesterol/lipid-phosphorus, and phospholipid/free cholesterol of the two types of subjects were significantly different (Groups A–C). It is notable that in the two groups, although the absolute concentrations of these lipid components were similar, the rela-

TABLE 2. RELATIONSHIP OF CERTAIN PLASMA LIPID FRACTIONS IN POSTABSORPTIVE NORMAL AND EPILEPTIC INDIVIDUALS, BEFORE, AND SIXTY MINUTES AFTER 0.4 MG. OF EPINEPHRINE INTRAMUSCULARLY

Ratios	Normal subjects		Epileptics		"P" values of differences between groups:			
	Controls (8)* A	Treated (6) B	Controls (10) C	Treated (10) D	A-C	A-B	C-D	B-D
F.C./T.C.† ×100	26.7‡ ± .9	27.5 ±1.3	29.0 ± .5	28.4 ±.7	ns§	ns	ns	ns
T.C./L-P	25.2 ±1.5	19.9 ±2.0	31.0 ±1.6	24.4 ±.7	.04	ns	<.01	ns
Plp./F.C.	3.97 ± .20	5.13 ± .56	2.97 ± .14	3.80 ±.16	<.01	ns	<.01	.03
N.F./Plp.	0.71 ± .12	1.51 ± .25	0.85 ± .11	0.51 ±.05	ns	.03	ns	.02

* Figures in parentheses are numbers of subjects in each group.

† Abbreviations: F.C. = free cholesterol; T.C. = total cholesterol; L-P = lipid-phosphorus; Plp. = phospholipids; N.F. = neutral fat.

‡ Mean ± S.E.

§ Non-significant.

tionships were different. Comparison of the mean values in the normal and epileptic groups after epinephrine injection (B–D) showed that the ratio phospholipid/free cholesterol was significantly lower than the comparable value in the normal group, and the ratio neutral fat/phospholipid of the epileptic group was significantly lower than the comparable value in the normals. It is also apparent from an inspection of the result-statistics shown in columns A–B and C–D that epinephrine administration affected differently the interrelationships of the plasma lipid components in the normal group compared to the epileptics. Reference to Table 1 shows that the differences in these lipid relationships between the two groups of subjects in response to epinephrine were primarily due to the increased phospholipid concentration in the epileptics, and the increased absolute concentration of the neutral fat in the normal subjects.

DISCUSSION

Several investigators have reported that there was no difference in the levels of plasma lipids between normal and epileptic subjects. McQuarrie

et al. (9), however, reported that the cholesterol/lipid-phosphorus ratio in epileptic children was significantly higher than in normals. Peters and Van Slyke (10; p. 518) claimed that such a difference between normal and epileptic subjects would be expected, since the average cholesterol values in the former group were lower than in the epileptics. In our study no differences were found in the concentrations of the plasma lipid components in the two types of patients, but the ratio total cholesterol/lipid-phosphorus was significantly higher in the epileptics compared with normal subjects. An inspection of the data in Table 1, columns A–C, shows that this difference in the plasma lipid interrelationships in the two types of subjects was valid, and not due to differences of the absolute concentrations of cholesterol or phospholipids. Furthermore, the coefficients of variation or "spread" of the concentrations of these two components in normal and epileptic subjects were found to be small and quite comparable for the two groups; in the normals the values were 9.8 and 14.8 per cent for phospholipids and total cholesterol, respectively, and in the untreated epileptics the coefficients of variation were 16.8 and 19.6 per cent, respectively.

The administration of epinephrine resulted in a marked increase in the plasma level of phospholipids in the epileptics, whereas the increase was in the level of the neutral fat component in the normals. The higher neutral fat concentration in the latter was most likely due to the mobilization of fat from the depots in response to an increased metabolic need. The increased phospholipid concentration without change in the neutral fat component, found in the epileptics, differentiates them from the normal subjects in their type of response to epinephrine. It is generally believed that the phospholipids of the blood are formed in the liver and removed from the blood by the same organ. The available data suggest that there is an increased synthesis of liver phospholipids whenever fat metabolism is increased, for example, when carbohydrate breakdown in the liver is deficient. In relation to the present data, it is therefore of interest that in our earlier studies (2) one of the principal findings was a "subnormal" glycemic pattern during the one hundred and twenty minutes after hormone administration.

It is generally believed that the ratio values derived from the concentrations of certain plasma lipid components are more sensitive indicators of quantitative changes in lipid metabolism than are the absolute concentration values. In normal subjects such interrelationships have been shown to be much more constant than those of any of the lipid fractions from which they were derived. In Figure 1 the ratios lipid-phosphorus-3.62/total cholesterol and free cholesterol/total cholesterol were plotted as suggested by Peters and Van Slyke (10; p. 472). The points for normal subjects

should fall in rectangle 5. This was the case for most of the normal group in this study, before and after epinephrine administration. On the other hand, the points for 8 out of 10 epileptic controls were in rectangle 8. Peters and Van Slyke state that in most of the few pathologic cases which have been found to fall in rectangle 8, the total cholesterol level was below 100 mg. per 100 ml. The total cholesterol concentration in our epileptics

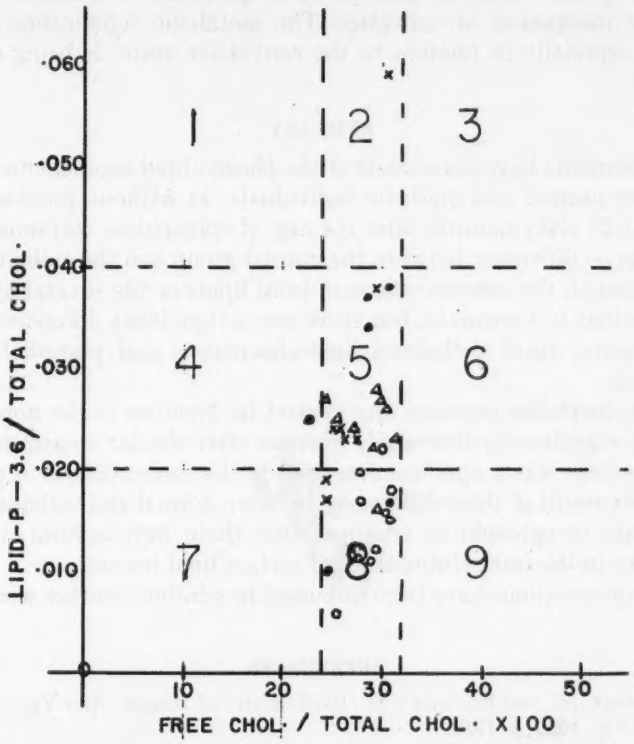

FIG. 1. The interrelationships of plasma lipids in postabsorptive normal (x) and epileptic individuals (o) without treatment; and sixty minutes after 0.4 mg. of epinephrine intramuscularly: (•) and (△), respectively.

was in the neighborhood of 200 mg. per 100 ml.; and the average was not different from that of the normal group. Thus, the lipid interrelationships in epileptics appear to be unique and have not been observed in any other pathologic condition. After epinephrine administration, 8 of 9 points were in rectangle 5, that is, in the rectangle characteristic of normal subjects. This shift in the location of the points was highly significant ($P = <.01$), and suggests that epinephrine administration changed the abnormal pat-

tern of lipid interrelationships in the epileptics to that observed in the normal group.

The present data and those of our previous study (2) show that the epileptic differs from the normal human both in the pattern of the plasma lipid relationships and in the types of responses of plasma components elicited by a general stimulus such as epinephrine. These differences are undoubtedly due to either qualitative or quantitative differences in the metabolic mechanism of epileptics. The metabolic significance of these findings, especially in relation to the convulsive state, is being explored further.

SUMMARY

Measurements have been made of the plasma lipid components in post-absorptive normal and epileptic individuals, 1) without previous treatment, and 2) sixty minutes after 0.4 mg. of epinephrine intramuscularly. There was no difference between the normal group and the epileptic group with respect to the concentrations of total lipids or the several lipid fractions previous to treatment, but there was a significant difference regarding the ratios total cholesterol/lipid-phosphorus and phospholipid/free cholesterol.

After epinephrine injection the neutral fat fraction in the normal subjects was significantly increased, whereas after similar treatment of the epileptics there was a significant increase in the concentration of phospholipids. As a result of these differences between normal and epileptic groups in response to epinephrine adminstration there were several significant alterations in the interrelationships of certain lipid fractions.

These observations have been discussed in relation to other work in this field.

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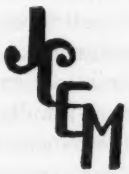

Editorial

SOME RECENT ADVANCES IN THE KNOWLEDGE OF HYPOGLYCEMIA

IN INFANTS and children, the early diagnosis and treatment of hypoglycemia is of extreme importance because at these ages there is great likelihood that the cells of the brain will be permanently damaged by the disease. Unfortunately, hypoglycemia occurring in infants and children always has been difficult to classify, to diagnose, and to treat. One of the most important of the recent contributions to the knowledge of hypoglycemia is McQuarrie's summary (1) of his significant contributions on this subject. In a study of 40 children having hypoglycemia, McQuarrie found that idiopathic spontaneous hypoglycemia was the most common (25 of the 40 patients). Other causative factors were adrenocortical failure, glycogen-storage disease of the liver, anterior-pituitary failure (dwarfism), hypothyroidism (cretinism), islet-cell tumor, and congenital galactosemia.

The etiology of idiopathic spontaneous hypoglycemia, which is a fasting and not a stimulative hypoglycemia occurring only in children, still is unknown, although McQuarrie noted familial incidence in 11 of the 25 patients. In biopsy specimens of the pancreas in 2 siblings, alpha cells were not found; however, in biopsy specimens of the pancreas in several other patients, alpha cells were found.

The hypoglycemia in each of the 25 patients with spontaneous idiopathic hypoglycemia was controlled by the administration of minimal maintenance doses of corticotropin. The condition spontaneously disappears as the child grows older. McQuarrie points out that consistently good results in alleviating hypoglycemia have not been obtained from treatment with diet, alloxan, or subtotal pancreatectomy.

Underdahl, Woolner and Black (2) recently reviewed the association of functioning islet-cell adenoma with functioning and nonfunctioning adenomas in other endocrine glands such as the parathyroid, pituitary, and adrenal, and described a series of 8 such cases. These investigators emphasized the diagnostic importance of determining serum calcium and phosphorus levels and of obtaining roentgenograms of the sella turcica in any patient with a functioning islet-cell tumor.

A distinction probably can be made between the occurrence of a few

encapsulated islet-cell adenomas in the pancreas and the occurrence of nodular hyperplasia (adenomatosis) in which both nonencapsulated and encapsulated collections of cells resembling beta cells are located diffusely throughout the entire pancreas and may be associated with a similar pathologic entity in the parathyroid. Among several of our patients (3) having that type of nodular hyperplasia, 2 were treated by resection of a third of the pancreas. Postoperatively each showed permanent diabetes mellitus, instead of the temporary type that usually occurs after such an operation. A similar case also was noted by Frantz (4). The development of permanent diabetes in these instances suggests that in patients having nodular hyperplasia, the remaining normal islet-cell tissue perhaps is replaced largely by these multicentric neoplasms which are nonfunctioning. In a recent series (5) of 65 patients having single or multiple islet-cell tumors who were surgically treated at the Mayo Clinic, permanent diabetes developed in only 2—one had had a total pancreatectomy and the other, a subtotal resection of the head of the pancreas.

In each of our 2 previously mentioned patients having nodular hyperplasia of the islet cells of the pancreas, a preoperative diagnosis of asymptomatic hyperparathyroidism was made, based on results of laboratory studies; the diagnosis was proved at operation. Prior to partial pancreatectomy in one, repeated determinations showed a low concentration of serum phosphorus. After pancreatectomy, however, the concentration was normal, both before and after the operation on the parathyroids. Similarly, after the partial pancreatectomy in the second patient, the serum phosphorus values were normal, both before and after the operation on the parathyroids. The normality of the serum phosphorus values after partial pancreatectomy probably resulted from decreased phosphorylation of glucose that was caused by the decrease in insulin production. These values might have led to complications in the diagnosis of hyperparathyroidism had not the serum calcium levels remained elevated in each patient.

Several reports (6–9) have appeared in the literature which indicate that chronic hypoglycemia caused by functioning islet-cell tumor may result in damage to the anterior-horn cells of the spinal cord, as well as to the cells higher in the central nervous system. The neurologic syndrome described has been that of a progressive muscular atrophy with marked muscular weakness, atrophy, and fibrillations. Associated symptoms and signs due to the effects of hypoglycemia on the higher centers of the nervous system have been present in each of the patients described in the literature, thus facilitating the diagnosis of hyperinsulinism. In 1 case (8), proof of damage to the anterior-horn cells was obtained at postmortem examination. In 1 patient (6) this syndrome developed one month after removal of the islet-cell tumor. In all of the patients, successful removal of the functioning

adenoma resulted in eventual recovery, although in 1 patient (7) there was some residual atrophy.

A rare type of functioning islet-cell tumor simulating sarcoma recently has been described (10) with detailed clinical and pathologic studies of 2 cases and a review of 3 other similar cases previously reported as sarcomas in the literature. Such tumors are large and most frequently are located outside of the pancreas in the abdomen and chest. A large tumor was removed from each of the 2 newly described patients; no recurrence of the tumor or of hypoglycemia had been noted, two and three years, respectively, after operation. Microscopic examination revealed each tumor to be composed of round cells without fibrils simulating beta cells and spindle-shaped cells with cytoplasmic processes simulating spindle-shaped sarcoma cells. These tumors are probably insulin-producing, low-grade islet-cell carcinomas.

PENN G. SKILLERN, M.D.

2020 East 93rd Street,
Cleveland 6, Ohio

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Letter to the Editor

17-HYDROXYCORTICOID DETERMINATIONS IN SWEAT

To the Editor:

The response of the adrenal cortex to various types of stress is often studied by determination of the 17-hydroxycorticoid content of blood or urine. Many of these investigations involve athletic events in which blood sampling is not practical. For that reason, urinary determinations are more frequently used. Since many of these studies involve physical activity in which there is likely to be a large weight loss due to sweat, it is important to know whether sweat contains an appreciable amount of adrenal corticoids. If there is such a loss in the sweat, the value for urinary excretion of 17-hydroxycorticoids would indicate a lower level of adrenocortical activity than actually exists. A review of the literature fails to reveal any mention of work concerning the presence of adrenal corticoids in sweat. Accordingly, an experiment was designed to determine whether they are present in sweat collected during severe exercise, with and without an artificially induced high blood level of adrenocortical steroids.

METHODS AND MATERIALS

Sweat was collected during one hour of exercise from both forearms of a normal 25-year-old male subject on four days. The exercise consisted of running and calisthenics. The samples were collected in two polyethylene plastic bags which extended above each elbow. On two days urine was collected from the beginning of the exercise period until four hours later. On one of these days 25 mg. of hydrocortisone in 25 ml. of 10 per cent alcohol was injected intravenously five minutes before the beginning of the exercise. This dose was given in order to cause an increased blood level of 17-hydroxycorticoids for at least part of the collection period, because some stress studies involving athletic events had shown that exercise alone is not a sufficient stimulus to cause a significant increase in the sustained secretory activity of the adrenal cortex (1). The collected sweat was analyzed for 17-hydroxycorticoids by the method of Reddy-Jenkins-Thorn (2, 3), with the following alterations. After the butanol extract was decanted following the addition of sodium carbonate, 15 ml. of this butanol extract was evaporated to dryness and then dissolved in 1 ml. of absolute

alcohol, USP. Colorimetry was then carried out with a 0.3-ml. sample, using an ethanol blank and a standard of hydrocortisone dissolved in ethanol. The 17-hydroxycorticoid content of urine was assayed by the same method without these alterations.

RESULTS

By the Reddy-Jenkins-Thorn method there was no demonstrable 17-hydroxycorticoid in 3 of the sweat samples, but in the fourth, which was collected on a day without exogenous hydrocortisone, there was approximately 0.3 μg. of Porter-Silber-reactive chromogenic material per ml. of sweat. Twenty-five ml. of the same sample was then hydrolyzed with β-glucuronidase (300 units/ml.) for forty-eight hours at 37° C. and the extract was chromatographed on paper by the aqueous methanol-toluene system of Bush (4). It was found to contain no demonstrable adrenocortical steroids. The urinary steroid content on a day without administered hydrocortisone was 1.8 mg. for the four-hour period. Urine collected during the four-hour period on the day when hydrocortisone was administered intravenously contained 5.6 mg.

DISCUSSION

The Reddy-Jenkins-Thorn method is capable of measuring quantities of 17-hydroxycorticoid equal to, or greater than 1 μg. per ml. (5). Since the samples of sweat were concentrated fifteen times, this would indicate that in 3 of the samples the concentration of 17-hydroxycorticoids was less than 7 μg. per 100 ml. The fourth sample, which contained 30 μg. of a Porter-Silber-reactive chromogenic material per 100 ml., showed no demonstrable adrenal corticoids when assayed by paper chromatography. On the basis of the limitations of the methods involved, this would mean that the concentration of adrenal steroids in the sample of sweat was less than 8 μg. per 100 ml. The nature of this chromogenic material is unknown. It is significant that the sweat collected on the day of hydrocortisone administration contained no measurable 17-hydroxycorticoids, in spite of a threefold increase in the urinary excretion of 17-hydroxycorticoids in this period induced by the intravenous injection of hydrocortisone.

SUMMARY AND CONCLUSION

Experiments have indicated that the concentration of 17-hydroxycorticoids in sweat collected from a normal subject during severe exercise was less than 8 μg. per 100 ml., both on control days and on a day when 25 mg. of hydrocortisone was injected intravenously immediately prior to exercise. On this basis it seems justifiable to conclude that in situations of stress which involve severe physical activity, with concomitant loss of

large amounts of sweat, negligible quantities of adrenal corticoids are excreted by this route.

J. L. LEWIS, JR.*
GEORGE W. THORN, M.D.

Department of Medicine,
Peter Bent Brigham Hospital and Harvard Medical School,
Boston, Massachusetts

* Second year student, Harvard Medical School.

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E. KOST SHELTON, M.D.
1888–1955

Eberle Kost Shelton, secretary (1935–1941) and president (1942–1944) of the Endocrine Society, died February 22, 1955 in Santa Monica, California following an operation for intestinal obstruction due to diverticulitis of the colon.

Dr. Shelton was born in Bloomfield, Iowa, May 9, 1888 and received his M.D. degree from the University of Colorado Medical School in 1911 (Honorary D.Sc., 1944). After finishing his formal education, he practiced in the frontier town of Antonito, Colorado for sixteen years, moving to Santa Barbara, California in 1929 and to Los Angeles in 1936.

In 1927 Dr. Shelton met, and later collaborated with the late Dr. William Engelbach in the publication of a four-volume basic work entitled "Endocrine Medicine" (1932). The then uncharted field of the ductless glands was a great challenge to his imagination, and the many original articles published by Dr. Shelton in medical journals, text books and medical reference books attest to his keen clinical insight and far-reaching vision.

In 1930 he founded The Shelton Clinic in Santa Barbara. Later he moved the Clinic to Los Angeles, and was its director until his death. To his associates he was a great inspiration and leader, a superb teacher, sound advisor, and close friend.

Dr. Shelton gave generously of his time and energies in the investigation and treatment of the sick, and especially enjoyed sharing his knowledge with others. He was an illustrious clinician and keen observer with a propensity for teaching. He served as director of The Endocrine Clinic, City and County Hospital of Los Angeles, 1931–1954; Associate Clinical Professor of Medicine, University of Southern California Medical School, 1931–1953; and Clinical Professor of Medicine, University of California Medical School at Los Angeles, 1953–1955. He was also Attending Staff Member, Los Angeles County Hospital; Consulting Staff Member, St. John's Hospital, Santa Monica; Diplomate of the American Board of Internal Medicine; Fellow of the American College of Physicians; and a member of the Endocrine Society, the American Geriatrics Society, the Los Angeles Academy of Medicine, the American Medical Association, the California Medical Association, and the Los Angeles County Medical Association.

Dr. Shelton's quick wit and warm personality drew to him many friends and the admiration of all those who came in contact with him. His loss is deeply felt.

SHELDON PAYNE, M.D.
The Shelton Clinic

The Endocrine Society

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-SEVENTH MEETING

THURSDAY, FRIDAY AND SATURDAY, JUNE 2, 3 AND 4, 1955

Haddon Hall

Atlantic City, New Jersey

(Abstracts arranged according to numbers on the printed program)

THURSDAY, JUNE 2, 1955

9:30 A.M. THYROID

CHAIRMEN: R. W. BATES AND F. R. KEATING, JR.

1. Thyro-Pituitary Relations in Cretinism and Hypothyroidism.

*A. M. DiGeorge, M.D., S. A. D'Angelo, Ph.D.** and *K. E. Paschkis, M.D.** Division of Endocrine and Cancer Research, Jefferson Medical College and St. Christopher's Hospital for Children, Philadelphia, Pa.

Studies were made in cretinism, in adult hypothyroidism, and in ophthalmopathy. Assays of serum TSH levels (stasis tadpole method) were performed in addition to studies of various parameters of thyroid function. Serum TSH levels would be expected to be elevated in severe hypothyroidism, on the basis of a reciprocal action of thyroid hormone and TSH; elevated TSH levels are frequently observed in untreated hypothyroidism. However, in the cases selected for this study TSH levels were low, or zero. They rose significantly under thyroid medication. In an untreated cretin, aged 11 years, TSH first appeared after thyroid was administered. One obvious implication of these observations is that if TSH is absent or at a low level, this does not necessarily indicate pituitary origin of hypothyroidism ("pituitary myxedema"). Furthermore, it is apparent that under certain circumstances the thyroid hormone may stimulate, rather than suppress secretion of TSH. Stimulation may occur with doses of thyroid medication which are usually suppressive. Analysis of the cases suggests the possibility that the process of TSH synthesis and/or release may at some time require the presence of thyroid hormone, and that there may be considerable quantitative variation of this requirement.

2. Early Prediction of Success or Failure of Radioiodine Therapy of Hyperthyroidism.

Alvin Schultz, M.D., and Leslie Zieve, M.D. (Introduced by Edmund B. Flink, M.D.) Veterans Administration Hospital and University of Minnesota, Minneapolis, Minn.

Sixty-six hyperthyroid patients given 89 doses of radioiodine were evaluated before, and serially after therapy with respect to clinical status, thyroidal uptake, BMR and serum levels of cholesterol and chemical protein-bound iodine. Three groups—failure, remission, and myxedema—were defined clinically after adequate follow-up (averages,

* Member.

21, 77 and 79 weeks respectively). These were clearly distinguishable by the thyroidal uptake as early as six to nine weeks following therapy and at each examination thereafter. The PBI was as effective, BMR almost as effective, and cholesterol much less effective in separating failures from remissions. With one exception, a combination of variables was more effective than any single measure. Radioiodine therapy was a failure in 96 per cent of those who were hyperthyroid clinically and whose uptake and BMR were both either abnormally high or borderline at their first adequate post-therapy evaluation. Failure occurred in only 2 per cent of those who were (a) hyperthyroid clinically, with uptake and BMR both definitely normal, (b) borderline euthyroid clinically, with either uptake or BMR normal, or (c) definitely euthyroid clinically. As early as six to nine weeks, over half of the cases of remission or myxedema, but only one twentieth of the failures showed non-palpable glands. Later, group differences were even more striking.

3. A Method for Iodide Balance Studies in Animals on Low Iodide Diets.

*L. Van Middlesworth, M.D.** Department of Physiology, University of Tennessee, Memphis, Tenn.

Rats ate fixed amounts of diet per day, with 0.5 cc. of an I^{131} solution, *initially* containing 0.05 μc./0.5 cc. Urine and feces were collected daily and gamma-counted against a standard of one daily dose of I^{131}. The rats could accumulate no more than 0.23 μc. of I^{131} and no radiation effect was observed. After equilibration the sum of urinary and fecal I^{131} subtracted from the daily dose indicated the iodide balance. Rats fed a goitrogenic processed cereal diet (0.03 μg. I^{127}/Gm.) required ten to twenty days to establish I^{131} equilibrium. Each equilibrated rat contained 4 to 9 times the daily I^{131} intake; two thirds of the daily iodide excretion was fecal. The first day after propylthiouracil the urine contained one quarter of the total body I^{131}; three quarters of the body iodide was lost in ten days. Discontinuance of propylthiouracil was followed by fifteen days of positive iodide balance. Triiodothyronine (0.55 μg./day) or casein (25 per cent), unlike *l*-thyroxine (0.5 μg./day), added to the cereal diet increased urinary and decreased fecal excretion and resulted in positive iodide balance for two weeks. After discontinuing triiodothyronine the balance remained positive for several days, then was negative for two weeks. This tagged diet procedure offers the only available method to obtain these data.

4. Therapeutic Implications of the Avidity of Solitary Thyroid Nodules for I^{131}.

*Martin Perlmutter, M.D.**, and *Stanley L. Slater, M.D.* Maimonides Hospital, Brooklyn Veterans Administration Hospital and State University of New York College of Medicine at New York City

Solitary *nontoxic* thyroid nodules were classified as "hot" or "cold" depending upon whether or not their uptakes of I^{131} were more than that of a non-nodular area of thyroid tissue of the same patient. The following data suggest that patients with *nontoxic* solitary "hot" nodules may be spared an operation performed solely because of the possibility of carcinoma:

Type of nodule	*Pathologic diagnosis*		*Total number*
	Benign	*Malignant*	
"Cold"	65	22	87
"Hot"	32	0	32
			119

The ingestion of thyroid hormone significantly decreases the thyroid uptake of I^{131} in 95 per cent of euthyroid subjects and in only 3 per cent of thyrotoxic patients. Half of the *nontoxic* "hot" nodules and all of the *toxic* "hot" nodules failed to be significantly inhibited. Definite inhibition of the function of a nodule, therefore, indicates the euthyroid state; but lack of inhibition does not indicate hyperthyroidism in nodular goiter, as it does in Graves' disease. The prolonged ingestion of thyroid hormone failed to cause the *nontoxic* "hot" nodules to disappear, but some decreased in size. *Toxic* nodules should be treated surgically or isotopically.

5. Deiodination of Thyroxine to Triiodothyronine by Kidney Slices of Rats with Varying Thyroid Function.

Frank C. Larson, M.D., Kenkichi Tomita, Ph.D. and *Edwin C. Albright, M.D.** Department of Medicine and the Enzyme Institute, University of Wisconsin, Madison, Wis.

Deiodination of thyroxine to triiodothyronine by kidney slices of rats with varying thyroid function was studied. Rats were made hypothyroid by feeding thiouracil (0.3 per cent in the diet; 0.1 per cent in the drinking water) for four weeks. Others were made hyperthyroid by exposure to $-10°$ C, or by feeding 2 per cent desiccated thyroid, for eighteen days. Untreated rats served as controls. Chromatographically pure I^{131}-labelled thyroxine was added to surviving kidney slices in modified Krebs-Ringer phosphate solution. Following incubation the slices were homogenized, extracted with butanol, and thyroxine and triiodothyronine separated and identified by paper chromatography. Observations were made at 0, 3, 6, 9, 12 and 24 hours. There was virtually no deiodination in the thiouracil-treated animals. The significance regarding hypothyroidism is questionable, since inhibition resulted from addition of thiouracil in 3.1×10^{-6} molar concentration to normal slices. In the normal and hyperthyroid rats deiodination was most active during the first nine hours, with slight additional deiodination beyond twelve hours. At nine hours the normal kidney deiodinated 24 per cent of thyroxine; the hyperthyroid, 42 per cent. It is concluded that the thyroxine deiodinating system is conditioned by the level of thyroxine available in the tissues. This relationship is direct; hence the presence of an adaptive deiodinating enzyme is postulated.

6. Metabolism of Triiodothyronine and Thyroxine in Plasma, Pituitary and CNS of the Rabbit.

*J. Gross, M.D.**, *D. H. Ford, Ph.D.** and *M. Posner, B.S.* State University of New York College of Medicine at New York City

Male albino rabbits were injected intravenously with I^{131} labeled triiodothyronine or thyroxine and killed at various intervals after injection. Plasma samples were taken during the course of each experiment, and at its conclusion the brain and pituitary were removed, separated into their constituent portions and assayed. In some cases chromatographic analysis of the samples was carried out. After triiodothyronine the plasma radioactivity fell rapidly and at four hours was predominantly in the form of iodide. There was, on the other hand, a rapid entry of radioactivity into the pituitary and diencephalon—less rapid in other parts of the brain. Concentration of I^{131} was most marked in the posterior pituitary, and next in the anterior lobe, which attained respectively over 20 and 7 times the plasma I^{131} concentration at four hours. Most of the concentrated radioiodine was identifiable as triiodothyronine. After thyroxine the plasma radioactivity fell less rapidly and was predominantly as thyroxine after four hours.

Concentration of radioiodine was demonstrable only in the posterior pituitary (1.7 times that in plasma at four hours), the radioactivity being present mostly as thyroxine, with some detectable as triiodothyronine.

7. A Defect of Thyroidal Storage in Subacute (Giant-Cell) Thyroiditis.

*Sidney H. Ingbar, M.D.** and *Norbert Freinkel, M.D.** Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.; and the Thorndike Memorial Laboratory, Second and Fourth Medical Services, Boston City Hospital, and Harvard Medical School, Boston, Mass.

The pathophysiology of giant-cell thyroiditis was investigated in 9 patients with this disease. In all patients an augmented rate of turnover of glandular hormone was indicated by: 1) diminished or absent 24-hour thyroidal accumulation of I^{131}, 2) normal or increased concentrations of thyroxine-like or butanol-extractable I^{131} (BEI^{131}) in serum, and 3) abnormally early peak of BEI^{131} concentrations. Impaired retention of iodinated moieties other than thyroxine was indicated by: 1) abnormally large fractions of plasma radioactivity displaying the solubility characteristics of thyroglobulin (observations in 3 patients), and 2) unusually large discrepancies between concentrations of PBI^{127} and BEI^{127} in serum (observations in 2 patients). In 7 patients, within two weeks following completion of x-ray therapy, amelioration of symptoms was accompanied by return to normal of both thyroidal accumulation of radioiodine and time-concentration curves of BEI^{131}. Following remission, abnormal quantities of butanol-insoluble I^{131} could not be demonstrated in plasma. The foregoing observations suggest that an idiopathic subacute thyroiditis, organic iodinations occur, although their absolute rate cannot be quantified. The apparent increase in the rate of turnover of glandular thyroxine, coupled with the appearance of a thyroglobulin-like moiety in the circulation, may reflect a storage defect resulting from a disruption of follicular integrity. X-irradiation appears to reverse this defect.

8. Placental Transfer of Thyrotropic Hormone in the Rat.

Miroslava Nikitovitch, M.A. and *Ernest Knobil, Ph.D.** Department of Physiology, Harvard Medical School, and Biological Research Laboratory, Harvard School of Dental Medicine, Boston, Mass.

The influence of maternal thyrotropin on the fetal thyroid gland has been investigated. Pregnant rats were hypophysectomized or thyroidectomized on the twelfth day of gestation. Some of the hypophysectomized animals were given 0.1 per cent propylthiouracil (PTU) in the diet, starting the day of operation. In addition, intact pregnant rats were fed 0.1 per cent PTU from the twelfth day of pregnancy. Intact pregnant rats on Purina laboratory chow served as absolute controls. All animals were sacrificed on the twenty-first day of gestation; the fetuses were removed and their thyroid glands (pooled glands from 4 fetuses = one observation) weighed on a torsion balance. In two groups of pregnant rats, one hypophysectomized and the other intact, a dose of 20 μc. of I^{131} was administered twenty-four hours prior to autopsy and the radioiodine uptake of the thyroids determined. Hypophysectomy of the mother failed to influence fetal thyroid weight and I^{131} uptake. Thyroidectomy of the mother also was without effect on fetal thyroid weight. The administration of propylthiouracil to pregnant rats, however, effected a highly significant increase in fetal thyroid weight which was not prevented or modified by prior hypophysectomy of the mother. It was concluded that: 1) the in-

crease in fetal thyroid weight observed following PTU feeding to pregnant rats is mediated by the fetal pituitary-thyroid axis rather than by an increased secretion of maternal TSH, and 2) TSH of maternal hypophyseal origin does not cross the placental barrier.

9. A Clinical Appraisal of Radioiodine Tests of Thyroid Function.

William M. McConahey, M.D., Charles A. Owen, Jr., M.D.* and *F. Raymond Keating, Jr., M.D.** Mayo Clinic, Rochester, Minn.

I^{131} tracers were administered to 250 patients with varying thyroid diagnoses and 125 patients without thyroid disease. The categories studied included exophthalmic goiter, adenomatous goiter with hyperthyroidism, adenomatous goiter without hyperthyroidism, myxedema and thyroiditis. Classification of the condition of the thyroid was based upon clinical impression, basal metabolic rate, serum protein-bound iodine level, and (particularly) response to treatment, and was not contingent on results of the I^{131} tests being evaluated. The following I^{131} tests were compared; the collection by the thyroid in 6 and 24 hours, the excretion into the urine in 24 and 48 hours, the thyroidal iodide clearance, the conversion ratio, the thyroidal accumulation rate and the extrarenal disposal rate. After analysis of the results obtained, it was concluded that in the diagnosis of exophthalmic goiter the most precise test is the thyroidal iodide clearance, although the most practical test is probably the 6-hour or 24-hour collection by the thyroid. The conversion ratio was found to be an accurate test, but requires too large a dose of I^{131} for routine use. All I^{131} tests appear to be somewhat less useful in the diagnosis of myxedema or adenomatous goiter, with or without hyperthyroidism.

10. A Study of Endemic Goiter.

Marcel Roche, M.D., Francisco De Venanzi, M.D., Jorge Vera, M.D., Eduardo Coll, M.D.* and *Mario Spinetti Berti, M.D.* Institute de Investigaciones Medicas, Fundacion Luis Roche, Caracas and Universidad de Los Andes, Merida, Venezuela

In the county of Bailadores (Estado Merida) located in the Venezuelan Andes, 84.5 per cent of 718 adults examined and 83.0 per cent of 641 schoolchildren were found to have abnormally palpable thyroids. In the adult group, 55.8 per cent, and in the children 68.6 per cent of the abnormal thyroids were palpable only; and 44.2 and 31.4 per cent, respectively, were both visible and palpable. The 48-hour radioiodine uptake in 100 adults averaged 74 per cent, and in 28 children 79 per cent. The urinary excretion of radioiodine in the forty-eight hours following ingestion averaged 18 per cent of the dose administered. There was no statistical difference in the thyroid uptakes and the urinary excretions between the nongoitrous adult subjects and those with goiter. Potassium perchlorate, when given two hours after I^{131} administration in doses of 300 mg. or higher produced a definite decrease in thyroidal radioactivity in 11 of 13 goitrous subjects, but failed to produce this effect in 6 normal individuals from a region of iodine sufficiency, suggesting that part of the thyroidal iodine in endemic goiter is in the unbound state. Clinical, hematologic and psychologic studies revealed no difference between the goitrous and nongoitrous group. The basal metabolic rate tended to be low in most patients studied.

11. A Comparison of Iodine Depletion Methods for TSH Assay in the Chick.

*Robert W. Bates, Ph.D.** and *Jerome Cornfield, B.S.* National Institutes of Health. Bethesda, Md.

Two methods for bioassay of thyrotropin (THS), using iodine depletion of the thyroids of baby chicks, were compared. The method of Piotrowski, Steelman and Koch (*Endocrinology* **52**: 489, 1953) is based on depletion of I^{127}, whereas that of Gilliland and Strudwick (*Clin. Sci.* **12**: 265, 1953) is based on depletion of I^{131}. Chicks of the same breed but from different hatcheries had very different iodine concentrations in their thyroids. In the I^{127} depletion method it was found that the standard deviation, S. D., was about one fourth of the initial iodine concentration and that the slope of the dose-response curve indicated about three fourths of the initial iodine concentration. This means that lambda, the ratio of S. D. to slope, was approximately the same at all iodine concentrations. When the I^{127} concentration was high (300 μg. 1/100 mg. of thyroid), the I^{131} uptake was so low (<5 per cent) that the chicks were not suitable for I^{131} depletion assays, but were better for I^{127} depletion assays. Conversely, when the I^{127} concentration was low (50–100 μg. 1/100 mg. of thyroid) the chicks were not suitable for I^{127} depletion assays, but were better for I^{131} depletion assays because the I^{131} uptake was high (40–60 per cent). When chicks were fasted, the iodine concentration in the thyroid was unchanged during the four-day period of assay after hatching, *i.e.*, there was no evidence for endogenous TSH production. However, iodine depletion occurred if chicks were fed a low-iodine diet, unless thyroxine was given to inhibit endogenous TSH production.

2:00 P.M. HYPOTHALAMUS AND PITUITARY

CHAIRMEN: O. H. PEARSON AND G. SAYERS

12. Evidence of Separate Hypothalamic Centers Controlling Corticotropin and Thyrotropin Secretion by the Pituitary.

*Monte A. Greer, M.D.** and *Howard Erwin, A.B.* National Cancer Institute, Bethesda, Md.

Previous investigations have indicated that the hypothalamus controls the secretion of various pituitary hormones, including thyrotropin and corticotropin. Amphenone (1,2 bis (*p*-aminophenyl)-2-methyl-propanone-1-dihydrochloride) has been shown by Hertz *et al.* to cause marked adrenal and thyroid hypertrophy. They have also shown that adrenal hypertrophy can be selectively prevented by cortisone administration, without impairing the goitrogenic effect, and that thyroid hypertrophy can be prevented by thyroxine administration without impairing adrenal enlargement in the amphenone-treated animals (*Proc. Soc. Exper. Biol. & Med.* **77**: 480, 1951). Both effects are lacking in the hypophysectomized animal. In the present study, 200 mg. of amphenone/Kg. body weight was administered orally for ten to fourteen days to adult female Holtzman rats weighing 230–290 Gm. Bilateral anterior hypothalamic lesions just dorsal to the ventromedian nucleus or in the region of the median eminence, 0.5 mm. lateral to the midline, were made from one to three weeks previously in 22 animals. Destruction of these areas had been found previously to interfere with the secretion of thyrotropin. The goitrogenic response to amphenone was greatly reduced or absent in the animals with hypothalamic lesions, compared to 16 similarly treated controls, but adrenal hypertrophy was not significantly affected. This indicates that the hypothalamic areas controlling thyroid and adrenal hypertrophy are discrete.

13. Effect of Hypothalamic Lesions on Blood ACTH Levels and 17-Hydroxycorticosteroid Secretion Following Trauma in the Dog.

David M. Hume and *Don H. Nelson.** Naval Medical Research Institute,

Bethesda, Md., and the Surgical Research Laboratory, Harvard Medical School, Boston, Mass.

Hypothalamic lesions were made in 18 dogs. Adrenal-vein cannulations were performed under ether anesthesia twenty-three days to six months later. Adrenal-vein blood 17-hydroxycorticosteroid minute output was determined by the method described by Hume and Nelson (*Fed. Proc.* 13–73, 1954). In 14 of these experiments the blood ACTH level in response to trauma was determined as well by the method described by Nelson and Hume (*J. Clin. Endocrinol. & Metab.* **14:** 781, 1954). Adrenal-vein corticoids were determined on 19 normal control dogs by the same method, and blood ACTH levels were measured in 8 of these animals in paired experiments on the same assay animals used for the lesion dogs. It was found that animals with lesions in the anterior portion of the median eminence showed no detectable blood ACTH following operative trauma, whereas normal dogs showed levels between 3 and 8 milliunits/100 cc. of arterial blood. The output of 17-hydroxycorticosteroids in the lesion animals averaged 1.8 gamma/minute, compared with 12.5 for the normal dogs. Lesions in other areas of the hypothalamus produced depression of pituitary-adrenocortical activity, varying from fairly marked to absent. There was no uniform correlation between the presence or absence of diabetes insipidus and the presence or absence of normal pituitary-adrenal response.

14. Role of the Neurohypophysis in the Release of ACTH.

M. Saffran, Ph.D., A. V. Schally, B.Sc. and *B. G. Benfey, M.D.* (introduced by R. A. Cleghorn). Allan Memorial Institute and Department of Pharmacology, McGill University, Montreal, Canada

Rat anterior-pituitary tissue *in vitro* was used as a means of detecting substances that control the release of ACTH. The ACTH was measured by the increase in the production of corticoids by isolated rat adrenal glands (Saffran M. and Schally, A. V.; *Endocrinology* (in press) 1955). Rat neural tissue (brain cortex, hypothalamus, neurohypophysis) added, together with epinephrine or arterenol, to the anterior pituitary caused an increased release of ACTH, although the amine or the neural tissue alone did not have a significant effect. Neurohypophyseal tissue was the most active tissue tested. A purified preparation of vasopressin, but not oxytocin, also stimulated the release of ACTH. On further purification of the vasopressin, its ACTH-releasing activity was lost. The activity was recovered in an impurity in the original vasopressin preparation. The active substance has been isolated by paper chromatography.

15. Pitressin and Blood ACTH in Adrenalectomized Rats.

*George Sayers, Ph.D.** and *Rosalie Burks, B.S.* Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

Blood ACTH was determined by a direct transfusion technic as follows: The left adrenal of a hypophysectomized rat was removed for analysis of ascorbic acid. A 4-ml. sample of blood was drawn into a syringe from the abdominal aorta of an adrenalectomized rat and quickly transfused into the saphenous vein of the recipient hypophysectomized rat. The total time for withdrawal and transfusion was usually less than sixty seconds. One hour later the right adrenal was removed for ascorbic-acid analysis. Blood taken from the adrenalectomized rat two minutes after induction of ether anesthesia induced ascorbic-acid depletion equal to 121 ± 11; at fifteen minutes, 72 ± 25; and at thirty minutes, 57 ± 6. Reduction in blood ACTH with time is not due to depletion of

pituitary stores. Pitressin in a dose of 5 units given over five minutes increased blood ACTH in rats subjected to ether anesthesia for thirty minutes (138 ± 18 in Pitressin-injected rats; 54 ± 20 in ether controls). Pitocin (10 units) and epinephrine (5 μg.) did not influence blood ACTH under the same conditions. The results suggest that a neurohumoral mechanism concerned with ACTH discharge is exhausted after thirty minutes of ether anesthesia and that antidiuretic hormone (or some other factor in commercial Pitressin) can substitute for this neurohumor.

16. Changes in Blood ACTH under Various Experimental Conditions, Using the Cross-Circulation Technique.

A. Brodish and *C. N. H. Long.** Yale University, New Haven, Conn.

A cross-circulation technique, using the rat as the experimental animal, has been developed to determine the ACTH content of blood. A stable basal secretion of ACTH is maintained over a 12-hour period by the untreated, cannulated donor animal. However, under various conditions of stress, such as unilateral or bilateral adrenalectomy, sciatic-nerve stimulation, l-epinephrine administration, and laparotomy, elevated levels of ACTH are found in the blood of these animals. Furthermore, it has been shown that the high rate of ACTH secretion, after the application of a severe stress such as unilateral or bilateral adrenalectomy, can be maintained for approximately two hours, after which time the blood ACTH concentration gradually diminishes and disappears in six hours. Thereafter, a slow increase occurs, returning to normal values in about twenty-four hours. The disappearance of ACTH in the blood six hours after a severe stress is not due to an increased blood level of cortical hormones, since it occurs after bilateral adrenalectomy. Neither is this phenomenon due to exhaustion of pituitary ACTH, for if a second stress is applied at this time, marked elevation of the blood ACTH level occurs. The blood levels of other pituitary hormones may be studied by this procedure.

17. Increase in Circulating Red Cell Volume of Hypophysectomized Rats Injected with Plasma from Pregnant Rats.

A. N. Contopoulos, M.D., D. C. Van Dyke, M.D., M. E. Simpson, M.D. and *H. M. Evans, M.D.** Institute of Experimental Biology, and Department of Anatomy, University of California, Berkeley, Calif.

The newborn young of many mammals have a normal, or higher than normal erythrocyte count and hemoglobin concentration, and these values decrease immediately after birth, reaching minimum levels within a matter of days or weeks, depending on the species. The failure of the rat in this early neonatal period to respond to hypoxia with an increased erythropoiesis makes it improbable that intra-uterine hypoxia is the stimulus to red cell production at this age (*Proc. Soc. Exper. Biol. & Med.* **86**: 713, 1954). The assumption that a humoral substance produced in the mother passes the placenta and stimulates the fetal marrow was directly explored by testing the presence of erythropoietic stimulatory substances in the plasma of pregnant rats. Circulating red cell volume and hemoglobin were determined by the Fe^{59} method in anemic hypophysectomized rats injected with plasma from pregnant rats at the 15–17th day of gestation. It was found that animals injected with plasma from pregnant rats showed a 30 per cent increase in circulating red cell volume and hemoglobin over those injected with plasma from normal rats or those injected with saline.

18. Influence of Growth Hormone and Cortisol upon Blood Formation in the Hypophysectomized Rat.

*Albert S. Gordon, Ph.D.** and *George J. Fruhman, Ph.D.* Department of Biology, Graduate School of Arts and Science, New York University, New York, N. Y.

Male rats, hypophysectomized four to five months previously, were injected daily for fourteen days with either: a) cortisol (1.0 mg.), b) growth hormone (GH, 500 μg.), c) a combination of cortisol and GH, or d) the vehicles for these hormones. **I.** *Peripheral Blood.* GH evoked an increase in plasma volume (T-1824 space), total RBC volume and reticulocyte count. Cortisol alone, or in combination with GH, elevated the red cell count, hematocrit percentage and hemoglobin concentration. Cortisol also resulted in eosinopenia, lymphopenia and neutrophilia, effects not influenced by simultaneous administration of GH. GH alone did not significantly affect these white cell parameters. **II.** *Bone Marrow.* A method was employed for estimating the absolute numbers of marrow cells (Fruhman and Gordon, 1954). GH produced an increase in marrow cellularity, involving increases in the total numbers of nucleated red cells and in those erythroid elements exhibiting mitoses. Cortisol treatment resulted in a decrease in the numbers of eosinophils, lymphocytes and myeloid elements of the marrow, whereas the numbers of nucleated red cell were unaffected. GH failed to prevent the depressive effects exerted by cortisol. **III.** *Splenic Erythropoiesis.* GH produced a striking increase in the numbers of splenic nucleated red cells. This effect was not evident in rats given cortisol alone, or in combination with GH.

19. Analysis of the Polyuria Produced by Hypophysectomy in Man.

M. B. Lipsett, M.D., J. P. Maclean, M.D., M. C. Li, M.D., C. D. West, M.D., B. S. Ray, M.D. and *O. H. Pearson, M.D.** Sloan-Kettering Institute, New York, N. Y.

Variations in water exchange following hypophysectomy for metastatic cancer have been reviewed in 41 cases. The completeness of hypophysectomy was indicated by disappearance of pituitary gonadotropin, low levels of thyroid function (serum protein-bound iodine, thyroidal radioactive iodine uptake), and inability to withstand cortisone or ACTH withdrawal. When autopsy examination of the sella was made, confirmation was obtained. With the foregoing criteria, it appears that polyuria does not depend upon the presence of the anterior pituitary. Clinical polyuria was present in 60 per cent of all cases, and in 67 per cent of those with complete hypophysectomy. In patients with minimal polyuria, a defect in urinary concentrating ability was still demonstrable. Polyuria was not seen when the pituitary stalk remained intact. These data illustrate the similarities of the hypothalamic-pars nervosa system in animals and man, and suggest that polyuria depends, in part, upon some remaining functional elements of this system following surgery. Hypertonic saline tests and standardized "maximal" osmotic U/P ratios were performed. Results of the hypertonic saline test are not always abnormal in the presence of polyuria. The "maximal" osmotic U/P ratio shows marked changes following hypophysectomy.

20. The Nature of the Effect of Alkali on Intermedin.

*M. S. Raben, M.D.** Department of Medicine, Tufts College Medical School, and the New England Center Hospital, Boston, Mass.

The concurrent processes of potentiation, prolongation of action, and inactivation of intermedin by alkali have been studied, using Oxycel-purified pituitary extract and the derived predominantly-intermedin and predominantly-corticotropic metabolic frac-

tions. Potentiation by 0.1N NaOH at 100° C. was greatest in the latter fraction, with a potency increase of from ten to thirty fold; it occurred within one minute, was maximal within three minutes, and fell (inactivation) to 1/3 maximal in thirty minutes and 1/10 maximal in one hundred and eighty minutes. Prolongation of action was also apparent within one minute, but increased progressively with the three- and thirty-minute treatments. Duration of intermedin action was increased perhaps 20 times by thirty- to sixty-minute treatment. Intermedin activity of untreated pituitary extract was readily destroyed by crude extracts of frog and rat liver and by purified trypsin and chymotrypsin, but the activity of alkali-treated material was resistant to inactivation by these substances. It is suggested that resistance to enzymatic inactivation accounts for the prolongation effect, and that the most likely alkali-induced molecular change to cause resistance to enzymes is racemization. Polarimetric measurements indicated progressively decreasing optical activity with hot alkaline treatment, the decrease being evident as early as three minutes.

21. Effect of Partial Hypophysectomy on Adrenocortical, Thyroidal and Gonadal Function in the Dog.

*William F. Ganong, M.D.** and *David M. Hume, M.D.* Surgical Research Laboratory, Harvard Medical School, Boston, Mass.

Varying amounts of pituitary tissue were removed from 31 dogs. Six to nine weeks later, they were sacrificed and sections of the pituitary area, thyroid, adrenals and gonads were examined. In 25 dogs, thyroidal I^{131} uptake and eosinopenic stress response were measured; 18 dogs were tested for compensatory adrenal hypertrophy, 9 had peripheral blood corticoid determinations before and after stress, and 6 had determinations of their adrenal venous corticoid output. Removal of approximately four fifths of the anterior lobe produced no endocrine abnormality detectable by these tests (6 dogs). With increasingly complete removal, gonadal function was first depressed (3 dogs), then thyroidal function (3 dogs), and finally adrenocortical function. No exceptions to this sequence and no isolated thyroidal or adrenocortical depression were seen. Cortical atrophy was the first evidence of adrenal depression. Unlike dogs with hypothalamic lesions, loss of stress responsiveness was never seen without concurrent adrenocortical atrophy. On the other hand, minute pituitary remnants were capable of supporting significant "compensatory hypertrophy" (7 dogs), adrenal venous corticoid output (3 dogs), and peripheral venous corticoid rises with stress (1 dog), despite well-marked adrenocortical atrophy. Complete depression of adrenocortical activity (5 dogs) was present only when the pituitary was completely removed.

22. Effect of Growth Hormone on Plasma Urea, Urea Excretion, Renal Function and Fasting Blood Glucose.

*Herbert Gershberg, M.D.** and *Joachim Gash, M.D.* Departments of Medicine and Physiology, New York University College of Medicine, New York, N. Y.

A dose of 3 mg./Kg./day of growth hormone injected for three days lowered the plasma urea concentration of 4 normal dogs from 32 mg. per 100 ml. to 15 mg.; from 48 mg. to 11 mg.; from 21 mg. to 7 mg.; and from 32 mg. to 24 mg. per 100 ml. The glomerular filtration rate increased 26 per cent, the renal plasma flow 28 per cent and sulphate Tm, 60 to 200 per cent. The excretion of urea decreased, but not to the same extent as the plasma urea concentration, with the result that the urea clearance increased 25 to 46 per cent. The latter effect was due primarily to the increased glomerular

filtration of urea. In 3 of the dogs, the fasting blood glucose level increased to 119 mg., 137 mg., and 146 mg. per 100 ml., respectively. In the fourth dog there was no increase in the blood glucose level, although the renal functions increased. Renotropic effects were obtained with even smaller doses of Wilhelmi preparation (1 mg./Kg. for two days), in comparison with which a Raben preparation was less active. Since urea constitutes a large part of urinary nitrogen, it is suggested that changes in glomerular filtration rate may participate in the transient shifts in nitrogen excretion seen in intact animals treated with hormones that have a renotropic effect.

23. Carbohydrate Metabolism in Intact, Hypophysectomized and Partially Pancreatectomized Rhesus Monkeys: Influence of Growth Hormone and Cortisone.

*Ernest Knobil, Ph.D.** and *Roy O. Greep, Ph.D.** Department of Physiology, Harvard Medical School and Biological Research Laboratory, Harvard School of Dental Medicine, Boston, Mass.

Hypophysectomy in the rhesus monkey results in inability to withstand fasting, increased glucose tolerance and increased sensitivity to insulin. The administration of beef growth hormone (Armour), 5 mg. per Kg. per day for five to seven days, failed to influence the foregoing parameters. In some instances, insulin sensitivity and glucose tolerance were increased by this growth hormone regimen. The administration of physiologic doses of cortisone acetate (3 mg. per Kg. per day) to hypophysectomized monkeys, however, returned insulin sensitivity and glucose tolerance to the normal range. The administration of beef growth hormone (5 to 10 mg. per day for two to three weeks) to subtotally pancreatectomized monkeys (*ca.* 80–90 per cent of pancreas removed) did not cause hyperglycemia or exacerbation of hyperglycemia if present. Cortisone acetate (5 mg. per Kg. per day) given for one week was also without effect on fasting blood glucose concentration. The acute effects of beef (Armour) and pig (Raben-Westermeyer) growth hormone (20 mg. per Kg. intravenously) were investigated in fasted, normal anesthetized monkeys. These large doses of growth hormone failed to produce consistent alterations in blood glucose levels. The blood levels of inorganic phosphorus and amino-acid nitrogen, as well as urinary nitrogen excretion, likewise showed no marked or consistent deviation from those of the controls.

24. Method for Measurement of Growth and Replacement of Cells in Man.

*James B. Hamilton, Ph.D.** State University of New York, College of Medicine at New York City

A reliable index of growth and replacement of tissues throughout the lifespan, rather than simply in childhood, would permit study of many problems. Growth rates of integumentary structures can be measured with some accuracy and appear to reflect certain body-wide states. Nails were selected as the integumentary appendage of choice. Studies were made of 300 male and 298 female Japanese, 3 to 88 years of age, including identical twins and members of large families; and of 500 male and 250 female Caucasians, 5 to 87 years of age, including identical twins. In mature individuals growth rates declined progressively and materially with age. Growth was more rapid during the day than during the night. The rate of growth tended to be more similar in siblings, and especially in identical twins, than in unrelated subjects of similar age.

FRIDAY, JUNE 3 1955,

9:00 A.M. REPRODUCTION AND SEX HORMONES

CHAIRMEN: R. O. GREEP AND L. WILKINS

25. Chorionic Gonadotropin Titers, Using *Bufo americanus* and *Bufo marinus*.

Edward H. Hon, M.D., and *John McL. Morris, M.D.* (introduced by C. Lee Buxton, M.D.). Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Conn.

Using the male toad as a test animal, a rapid practical method of estimating urinary chorionic gonadotropin titers can be established, provided: 1) the seasonal sensitivity of the toad and other factors which may affect response are known, and 2) a rapid method of hormone concentration is available for low levels or dilute urine specimens. The sensitivity of a number of species of toads, including *Bufo americanus* and *Bufo marinus*, was studied and dose response curves drawn up. Modification of the kaolin adsorption method by the use of a buffer and a new type filter reduced the time required for concentration to about ten minutes. Only 4 toads were required to ascertain whether the gonadotropin titer was outside the limits of normal pregnancy and the results were usually available in two to five hours. In over 2,000 tests for normal pregnancy no false positives were noted, and the problem of false negatives was eliminated by use of the concentration technique. In disturbances of pregnancy, the titering system proved extremely helpful in prognosis. Some of the patients with normal or low-normal values aborted, but all of the patients aborted who had values of less than 3,000 I.U. per twenty-four hours from days 50 to 90 of gestation.

26. Congenital Gonadal Dysgenesis, Ovarian Agenesis, and Male Pseudohermaphroditism: The Relationship to Theories of Human Sex Differentiation.

*Melvin M. Grumbach, M.D., Judson J. Van Wyk, M.D.** and *Lawson Wilkins, M.D.** The Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Md.

The relative role of the sex chromosomes and the embryonic gonads in the differentiation of the genital ducts and external genitalia has been the subject of considerable study. The hormonal theory of Wiesner (*J. Obst. & Gynaec. Brit. Emp.* **41**: 867, 1934; **42**: 8, 1935) and the finding by Jost (*Arch anat. micr. et morph. exper.* **36**: 271, 1947) that early fetal gonadectomy leads invariably to female development of the ducts and genitalia emphasizes the importance of the embryonic testes in counteracting the inherent tendency of the fetus to feminize. The sex chromatin pattern in skin biopsy specimens of 17 patients with the syndrome of ovarian agenesis have been studied and found to be male in 15. Such cases should be termed congenital gonadal dysgenesis. The technic is of considerable value in establishing the diagnosis at an early age. In addition 2 atypical cases have been studied in which there was development of Leydig cells and other rudimentary male components of the gonads without female components. These patients represent transitions between gonadal dysgenesis and male pseudohermaphroditism.

27. Urinary Excretion of Interstitial-Cell Stimulating Hormone (ICSH) by Women with Normal and Abnormal Menstrual Function.

*Janet W. McArthur, M.D.,** *Francis M. Ingersoll, M.D.* and *Jane Worcester, Ph.D.* Department of Gynecology, Harvard Medical School, Boston, Mass.

Single-dose assays for the presence of interstitial-cell stimulating hormone (ICSH) in the urine have been conducted daily throughout the menstrual cycle, employing the weight of the ventral prostate of the hypophysectomized male rat as the indicator. A total of 17 cycles in women have been so studied. Although the cycles of these individuals have differed greatly in length, the excretion pattern has shown surprising constancy with respect to other physiologic parameters. Peak rates of excretion are observed during two phases of the cycle. The first, greater in magnitude, begins abruptly twenty-four to forty-eight hours after the mid-cycle shift in basal body temperature, continues at a high level for the next one or two days and then declines over the next two to three days. The second begins in the late luteal phase and continues into the menstrual period. Repeated studies of the same individual indicate that the excretion pattern differs little from cycle to cycle. Similar longitudinal studies of the excretion pattern have been conducted in women with gynecologic disorders such as metropathia hemorrhagica, psychogenic amenorrhea, anovulatory sterility and the Stein-Leventhal syndrome. Significant differences from the normal pattern, either qualitative or quantitative, or both, have been found.

28. Function of the Postmenopausal Ovary: Urinary Estrogen Excretion and the Response to Administered FSH.

C. Alvin Paulsen, M.D., Robert B. Leach, M.D., Hershel Sandberg, M.D.* and *W. O. Maddock, M.D.** Wayne University College of Medicine, Detroit, Mich.

To ascertain the degree of ovarian function after the menopause, the urinary estrogen excretion of 51 postmenopausal women was compared to that of 21 menstruating and 23 castrated women. Urinary estrogens were determined by the immature rat uterine method. The estrogen excretion of the normal women ranged from 0.9 to 4.5 microgram equivalents of estradiol benzoate per twenty-four hours. None of the castrated women had estrogen levels above 0.3, whereas, 20 of the postmenopausal women exhibited estrogen levels above 0.3 (maximum 1.4). When these patients were classified according to "number of years postmenopausal," the average urinary estrogen excretion was found to be constant. Urinary estrogens fell to castrate levels within three months after ovariectomy in 3 postmenopausal patients studied before and after surgery. Hog FSH was administered, intramuscularly, in doses of 25 mg. twice daily for three weeks to 9 postmenopausal patients. Urinary estrogens increased five to seven fold during therapy in 3 patients. No increase occurred in the remaining 6 patients and in 2 castrated women used as controls. It is concluded that in many women the ovary continues to function at a low but significant level after the menopause and in some instances can be stimulated by administered gonadotropin.

29. Eunuchoidism with Negative Follicle-Stimulating Hormone in the Female.

M. P. Moldawer, M.D., F. Albright, M.D., P. H. Benedict, M.D.,* A. P. Forbes, M.D.** and *P. H. Henneman, M.D.** Massachusetts General Hospital, Boston, Mass.

Four cases are presented of a syndrome in the female characterized by eunuchoidism without other endocrine abnormality, and by a lack of follicle-stimulating hormone (FSH). The lack of FSH suggests that the primary disorder is a specific dysfunction of the pituitary. That the primary disorder is not one of estrogen metabolism is indicated by a normal action on the endometrium of estrogen and progestin. The findings in this syndrome are to be compared with eunuchoidism with negative FSH in the male. Both are lacking in FSH. They have one striking difference: in the male, chorionic

gonadotropin (APL) leads to testicular maturation, which may be complete after prolonged therapy. In the female the ovary shows no response, at least after up to eleven months of treatment. As evidence of lack of estrogen production in response to APL is the failure to induce progesterone-withdrawal bleeding after eight months of APL therapy. Other conditions are contrasted with this syndrome. Ovarian agenesis without dwarfism, and a rare condition of eunuchoidism with high FSH (in which the ovarian pathology is not clear) can be differentiated on the basis of a high FSH. When FSH is absent, non-virilizing arrhenoblastoma must be considered.

30. Prolactin as a Factor in Luetinizing Hormone Assay.

Albert Segaloff, M.D., Albert Flores, B.A.* and *Sanford L. Steelman, Ph.D.* Endocrine Research Laboratory, Alton Ochsner Medical Foundation, the Department of Medicine, Tulane University School of Medicine, New Orleans, La., and Biochemical Research Department, Armour Laboratories, Chicago, Ill.

The weight of the ventral prostate of the hypophysectomized immature male rat has been widely employed as a measure of luteinizing hormone (LH). It has been our experience that crude extracts tend to produce heavier prostates than more purified materials. Investigation of this problem has led to the finding that prolactin appears to sensitize the prostate to the action of LH. We have employed Sprague-Dawley rats, rested one or two weeks after hypophysectomy, treated for four days and sacrificed on the fifth day. A highly purified sheep LH (S.L.-227–80) preparation was employed in an amount sufficient to increase the ventral prostate by about 50 per cent. Preparations of prolactin from sheep and cattle were employed. They varied in potency from 7 to 35 I.U./mg. Prolactin in amounts of 0.1 to 10 I.U./day had little or no effect on ventral prostate or seminal vesicle weight. However, when amounts of 1 or more I.U. of prolactin were combined with the LH the increase in ventral prostate weight was more than doubled. The effects on testicular weight and seminal vesicle weight varied with the preparations. Thus, the addition of 1 I.U. of prolactin per day can introduce as much as a fivefold error in the estimation of LH activity by the ventral prostate method.

31. Effect of Depot Relaxin on Pubic Ligament Growth in Mice.

Bernard Kliman, A.B. and *Roy O. Greep, Ph.D.** Harvard Medical School and School of Dental Medicine, Boston, Mass.

The proliferation of pelvic ligaments during pregnancy in mice is under hormonal control. Similar ligament growth can be obtained experimentally by treatment of intact or castrated female mice with estrogen, followed by the injection of relaxin-containing extracts from the ovaries of pregnant sows. Previously, we have found that the most potent stimulus to ligament growth was not relaxin, but rather a more insoluble protein-like substance which was easily separable from relaxin but contained trace amounts of relaxin. Using the x-ray measurement of interpubic distance as an assay procedure, it was found that purified relaxin which is of low potency in mice can be enhanced by delaying its absorption from the site of injection. Of the injection media and binding agents which were tested, the most effective was a suspension of lyophilized relaxin in beeswax and oil, which produced a 65-fold increase in assay potency. In contrast, the mouse-active fraction of sow ovaries, which is notably less soluble than relaxin, was not potentiated by the depot medium. Control studies showed that the injection media alone were inactive. It was therefore concluded that the growth response of the pubic ligament can be obtained with depot relaxin, and that the mouse-active ovarian fraction represents a more slowly mobilized form of relaxin.

32. Fractionation of Relaxin by Chromatography and Counter-Current Distribution.

Edward H. Frieden, Ph.D., Matthew W. Noall, Ph.D. and *Francisco Alonso de Florida, M.D.* (introduced by William H. Fishman). Tufts College Medical School, Boston, Mass.

Considerable purification of relaxin-containing extracts from the ovaries of pregnant sows has been achieved by chromatography on ammonium Dowex-50. The most active fraction, eluted at pH 5.8–6.0, contains about 50 per cent of the original biologic activity together with about 3 per cent of the total protein. In the system sec-butanol-1 per cent TCA, the distribution coefficient for relaxin activity is approximately 1, as determined by assays following a 31-tube distribution. Distribution of the bulk of the protein present corresponds to a coefficient of 0.08 under these conditions. Both crude and partially purified relaxin preparations characteristically evoke a number of physiologic responses other than relaxation of the symphysis pubis (Frieden and Hisaw; *Recent Progr. Hormone Res.* **8**: 333, 1953). Our studies indicate that some separation of various activities may be achieved. The rat and guinea-pig uterine relaxation factors are concentrated in the relaxin-rich fraction from Dowex chromatograms. In distribution experiments, the mouse and guinea-pig uterine relaxation factors display partition properties very similar to those of relaxin. However, assays for the rat uterine-relaxing factor indicate that its properties are distinctly different, corresponding to a distribution coefficient of approximately 10.

33. Comparison of Oral and Subcutaneous Administration in the Anabolic and Androgenic Effectiveness of 19-Nortestosterone and Testosterone.

Dorsey E. Holtkamp, Ph.D., Arthur E. Heming, Ph.D.** and *Leo F. Mansor.* Research and Development Division, Smith, Kline and French Laboratories, Philadelphia, Pa.

19-Nortestosterone and testosterone were given to castrated male rats (6 per group) daily for seven days. With subcutaneous administration both agents, in doses of 1, 2.5, 5, 10 and 20 mg./Kg./day, produced progressively greater anabolic effects (greater mean weight of the levator ani muscles). Minimal doses necessary to produce levator ani muscle weight equivalent to that of the intact control animals were 7 mg./Kg./day of 19-nortestosterone and 11 mg./Kg./day of testosterone (interpolated). Comparable androgenic effectiveness (mean seminal vesicle weight 14 per cent that of intact controls) was obtained with 5 mg./Kg./day of 19-nortestosterone and 1 mg./Kg./day of testosterone. Orally, testosterone in doses of 5, 12.5, 25, 50 and 100 mg./Kg./day produced only very weak anabolic and androgenic effects. On the contrary, comparable oral doses of 19-nortestosterone produced definite and progressively greater anabolic responses bearing straight-line log-dose response relationship. About 60 mg./Kg./day (interpolated) was comparable in anabolic activity to 7 mg./Kg./day given subcutaneously. Furthermore, for a comparable anabolic response, 19-nortestosterone was even less androgenic after oral than after subcutaneous administration.

34. The Metabolic Effect of 19-Nortestosterone

William H. Baker, M.D., Philip H. Henneman, M.D., Billy Baggett, Ph.D.,* Lewis L. Engel, Ph.D.,* Dorothy Tibbetts, S.B.* and *M. Brown.* Medical Laboratories of the Collis P. Huntington Memorial Hospital at Massachusetts General Hospital, Boston, Mass.

Bioasay of 19-nortestosterone showed a marked anabolic effect with minimal androgenic activity, compared to testosterone. It was hoped that administration of this compound to human beings would have a similar effect. A patient with breast cancer metastatic to bone was studied for fifteen days and found to be in a slightly negative nitrogen and calcium balance and slightly positive phosphorus balance. A dose of 50 mg. of 19-nortestosterone was administered intramuscularly daily for four months. Initially nitrogen and phosphorus balances became positive; calcium balance remained unchanged; and there was retention of sodium and potassium. The calcium balance gradually became less negative and in four months was positive; nitrogen and phosphorus balances returned to pre-treatment levels. X-ray examination revealed recalcification of the bony lesions. There was a slow, progressive increase in facial hair with flushing and acne—androgenic effect. One patient with osteoporosis due to hydrocortisone therapy was studied to see if 19-nortestosterone could reverse the negative calcium balance. During administration of 19-nortestosterone, nitrogen and phosphorus retention occurred with no change in calcium. 19-Nortestosterone is a potent androgenic, anabolic hormone equivalent to testosterone propionate. It did not reverse the negative calcium balance caused by hydrocortisone in 1 patient.

35. Effect of Castration and Various Steroids on Individual Muscles of the Guinea Pig.

Charles D. Kochakian, Ph.D., Carol Tillotson, B.S.* and *Edith Dougherty, B.S.* Oklahoma Medical Research Foundation, Oklahoma City, Okla.

Male guinea pigs of the Hartley strain were castrated at 538 (508–574) Gm. body weight and killed, with normal controls, six, twelve and twenty-seven weeks later. The carcasses were frozen in sealed plastic bags until 48 different muscles were dissected. The decrease in rate of growth of the muscles by areas was: Head > neck > chest and back. Small, or no changes were seen in the muscles of the other areas of the body. The retractor peni showed no further growth after castration, as did the masseter and digastric muscles. The carcass weight was +1, −6, and −13 per cent that of normal controls. Thirty days after castration a single 15-mg. steroid pellet was implanted subcutaneously and the guinea pigs killed twenty-one days later. Testosterone (5.9 mg. absorbed in twenty-one days) produced increases of 10 per cent or more in 38 muscles; methyltestosterone (7.0) in 22; androstan-17β,1,3-one (1.5) in 16; androstane-3,17-dione (9.6) in 20; androstene-3,17-dione (7.9) in 4; androstane-3α,17β-diol (1.6) in 4; epiandrosterone (6.0) in 10; methylandrostane-3β,17β-diol (1.6) in 3; and methylandrostene-3β,17β-diol (0.4) in 2. Testosterone increased 34 muscles above normal but did not restore the size of the temporal, masseter or digastric muscles. Furthermore, some of the muscles which were increased by testosterone had not been affected by castration. The various steroids did not show parallel effects on the different muscles.

2:30 P.M. CORTISOL DERIVATIVES AND ALDOSTERONE

CHAIRMEN: E. H. VENNING AND L. T. SAMUELS

36. Inhibition of ACTH Activity in the Rat after Administration of 9-α-Halogenated Steroids.

Woodrow B. Kessler, Ph.D., Roger F. Varney, Ph.D. and *Aleck Borman, Ph.D.* (introduced by J. H. Leathem, Ph.D.). Squibb Institute for Medical Research, New Brunswick, N.J.

Differences between the ascorbic-acid concentrations in the adrenal glands of cold-stressed and non-stressed rats were used as an index of endogenous ACTH activity.

Treatment with 9α-fluoro-F acetate (FF), 9α-chloro-F acetate (CF), and F acetate (F) in doses ranging from 50 to 2,000γ indicated that the degree of ACTH inhibition was not directly related to the sodium-retaining or glycogen-depositing activity of these steroids. Inhibition of ACTH activity was proportionately increased by increasing steroidal dose; and equal weights of all three steroids inhibited adrenal responses to approximately the same degree. Decreases in adrenal ascorbate concentrations were noted in intact FF-treated rats, but this effect was not noted in animals treated with CF or F. Moreover, data from hypophysectomized rats given ACTH in lieu of a cold-stress indicated a minor inhibition at the adrenal level, which was less than that due to pituitary inhibition. Blood eosinophil counts, thymus weights, and body weights were also determined. Adrenocortical steroid administration proved FF to be the most potent inhibitor of body weight gain, whereas CF was least inhibitory. Use of involution of the thymus and eosinopenia as criteria of adrenocortical activity showed that the compounds tested exhibited the following pattern: FF was more effective than CF, which was more effective than F.

37. The Suppression of Endogenous Adrenocortical Secretion with 9-Alpha-Fluorohydrocortisone.

Alan Goldfien, M.D., Jacques Saint-Marc, M.D., Paul M. Beigelman, M.D.* and *William F. Ganong, M.D.** Department of Medicine, Harvard Medical School and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

9-Alpha-fluorohydrocortisone has proved to be especially suitable for therapeutic, diagnostic, and experimental studies of adrenal secretory inhibition, by virtue of its prolonged action and of its potency. The latter minimizes the quantity of steroid metabolites contributed by the diagnostic agent. The effect of 9-alpha-fluorohydrocortisone on the urinary excretion of 17-ketosteroids and 17-hydroxycorticoids has been studied in patients with normal and hyperfunctioning adrenals. In the normal male, inhibition has been detected with doses as small as 1 to 2 mg. given as a single dose daily. The elevated 17-ketosteroid excretion of female patients with mild virilism has been maintained with 2 mg. daily at, or below normal levels for as long as eight months. Two female patients with bilateral adrenal hyperplasia exhibiting predominantly features of the adrenogenital syndrome, but also excreting increased quantities of 17-hydroxycorticoids, showed evidence of marked inhibition of both adrenal excretory products with doses of 5 mg. and 25 mg. of 9-alpha-fluorohydrocortisone daily. In contrast, 4 patients with Cushing's syndrome showed no significant decrease in steroid excretion with 5, 6, 10 and 25 mg., respectively.

38. Comparative Effect of Metacortandracin 9-α-Fluorohydrocortisone, and Hydrocortisone upon ACTH Secretion in Man.

Herbert S. Kupperman, M.D., Meyer H. G. Blatt, M.D.* and *Hans Wiesbader, M.D.* Department of Therapeutics, College of Medicine, New York University-Bellevue Medical Center, New York, N.Y.

A series of well documented patients with the adrenogenital syndrome has been observed following administration of different cortisone and hydrocortisone preparations. The initial response of the ketosteroid excretion in these patients after a standard oral dose of cortisone was well established. The comparative effectiveness of the different adrenal steroids were then studied after oral administration by noting the degree of depression of ketosteroid excretion at different dose levels. The decrease was taken as

a measure of the ability of the steroid to inhibit the ACTH secretion. A quantitative relationship was established and it was felt that this technique provided an accurate means of assaying the pituitary inhibitory activity of these steroids in patients with adrenal hyperplasia. The data indicated that hydrocortisone is 25 to 30 per cent more active than cortisone, metacortandracin is more than twice as active as hydrocortisone, and 9-α-fluorohydrocortisone is the most active of all the preparations. No untoward effects were noted with any of the preparations used except for the 9-α-fluorohydrocortisone. A total dose of 3 mg. a day of this preparation produced edema in 5 patients with the adrenogenital syndrome. None of these patients showed evidence of cardiac insufficiency.

39. Distribution of Radioactive Hydrocortisone (Compound F) and Radioactive Corticosterone (Compound B) in Human Subjects.

*Avery A. Sandberg, M.D., Claude Migeon, M.D.** and *Leo T. Samuels, Ph.D.** Departments of Medicine and Biochemistry, University of Utah, College of Medicine, Salt Lake City, Utah

The distribution of 4-C^{14}-hydrocortisone and 4-C^{14}-corticosterone and their metabolites was compared in plasma and urine of 5 normal subjects after intravenous injection. Similar studies, plus biliary excretion, were undertaken in 4 patients. In another patient, 4-C^{14}-hydrocortisone concentration in liver, muscle and fat (surgically obtained) was investigated. Labeled corticosterone disappeared from the plasma more rapidly than labeled hydrocortisone. The highest concentrations of radioactivity of conjugated corticosterone were reached earlier (sixty minutes) and maintained at higher levels than those of hydrocortisone (one hundred and twenty minutes). The distribution of the injected compounds in urine and bile was studied by subjecting specimens to the following sequential procedures: chloroform extraction (free steroids), β-glucuronidase hydrolysis, continuous ether extraction, and acid-heat hydrolysis. Approximately 90 per cent of hydrocortisone appeared in the urine in forty-eight hours, contrasted to 75 per cent of corticosterone. Conversely, only 1.5 to 5 per cent of hydrocortisone appeared in the bile while 20 to 30 per cent of corticosterone was recovered in forty-eight hours. Following injection of hydrocortisone the highest concentration of radioactivity was found in the liver—two thirds conjugated, and the remainder free at one hundred and twenty minutes. Lower concentrations were found in fat and muscle.

40. Studies with a New Series of Steroids: Metacortandracin and Metacortandralone.

M. M. Pechet, Ph.D., M.D. and *F. C. Bartter, M.D.** National Heart Institute, Bethesda, Md.

Metabolic studies were carried out with the Δ^{14}-diene steroids, metacortandracin (Meticorten) and metacortandralone (Meticortelone) in Addisonian patients; cortisone and hydrocortisone were given during the same regimen for comparison. Animal studies suggested (Perlman and Tolksdorf: *Fed. Proc.* 1955) activities 3 to 5 times those of the latter compounds. Meticorten (ΔE) and Meticortelone (ΔF), 30 mg. daily, resulted in slight sodium loss as compared with marked sodium retention with cortisone, 150 mg. daily. Endogenous creatinine clearance was raised markedly with Meticorten and Meticortelone, and unaffected by cortisone. The characteristic transitory potassium diuresis on starting, and the retention on stopping medication, noted with cortisone and hydrocortisone, occurred with Meticorten and Meticortelone. This phenomenon was quantitatively less with Meticortelone than with Meticorten. Thirty mg. of Meti-

corten daily produced nitrogen loss. Inhibition of normal adrenal function, as shown by diminished 17-ketosteroid excretion, occurred with both steroids. The absorption and excretion of these steroids were studied. Fifty mg. of Meticorten resulted in a threefold increase in blood corticoid levels, persisting for six hours after oral, and for one and one half hours after intravenous administration.

41. Acute Renal Response to Aldosterone: Physiologic Basis for a New Assay Method.

Grant W. Liddle, M.D., Jerome Cornfield, B.S., Alfred G. T. Casper, B.A.* and *Frederic C. Bartter, M.D.** National Heart Institute, Bethesda, Md.

An aldosterone assay method has been devised, using adrenalectomized female dogs maintained on a constant, high-sodium diet. Aldosterone, methylene chloride extracts of human urine, and desoxycorticosterone produced rises in urinary K and falls in urinary Na which were maximal two to three hours after intravenous administration. All three materials showed linear log-dose response curves with the same slope. This was true for both Na and K effects, over a 32-fold dose range. The ratios of potencies were the same, whether estimated from Na or K effects. This permitted the use of a sensitive, empirical aldosterone index in evaluating assays. The method has a lambda value of about 0.25. Quantitatively, aldosterone was thirty times as active as desoxycorticosterone by weight. Cortisone and hydrocortisone, which produced no decrease of urinary Na, increased it at higher dose levels. Activity corresponding to that of 1 to 100 micrograms of aldosterone a day was found in the urine of normal, cardiac and cirrhotic subjects. Sodium deprivation produced a tenfold increase. In normal subjects ACTH, while producing a tenfold increase in urinary 17-OH corticoids, produced a twofold increase in urinary aldosterone-like activity; cortisone in large doses was without significant effect on urinary aldosterone-like activity.

42. Secretion of Aldosterone by Hypophysectomized Dogs.

Gordon L. Farrell, M.D., Elizabeth W. Rauschkolb, A.B. and *Simon Koletsky, M.D.* (introduced by George Sayers, Ph.D.). Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

Three male dogs were hypophysectomized via the oral approach. Three additional dogs were subjected to sham hypophysectomy and served as controls. The animals were fed a soft diet. Six days following hypophysectomy or sham-hypophysectomy the dogs were bled from the adrenal vein. The sellar region and the overlying brain of each hypophysectomized dog were sectioned and examined histologically for pituitary remnants. No pituitary tissue was found. The adrenal venous blood was extracted with chloroform and subjected to analysis by paper chromatography for the isolation of aldosterone and 17-hydroxycorticosterone. Average 17-hydroxycorticosterone secretion rates were as follows: sham hypophysectomized, 21.4 μg./Kg./hr.; and hypophysectomized, about 2.2 μg./Kg./hr. (approximately 10 per cent of the control rate). Average aldosterone secretion rates were as follows: sham-hypophysectomized, 25.6×10^{-2} μg./Kg./hr.; and hypophysectomized, 17.0 μg. $\times 10^{-2}$ μg./Kg./hr. (66 per cent of the control rate). These experiments are interpreted to indicate, (a) that the presence of the pituitary is not essential for the maintenance of a low rate of 17-hydroxycorticosterone secretion, and (b) that the rate of secretion of aldosterone remains at approximately two-thirds of normal following hypophysectomy. It is suggested that the secretion of aldosterone is regulated by a physiologic mechanism only partially interrupted by removal of the pituitary.

43. Studies on the Excretion of the Sodium-Retaining Corticoid.

E. H. Venning, Ph. D., J. C. Beck, I. Dyrenfurth,* and *C. J. P. Giroud.* McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

Further studies have been carried out to ascertain what factors are responsible for the changes in excretion of the sodium-retaining corticoid, aldosterone. When a dose of 600 μg. of aldosterone was administered intravenously to a bilaterally adrenalectomized patient, 79.8 μg. could be recovered in the urine. Seventy-seven per cent of the excreted mineralocorticoid was conjugated. Electrolyte balance studies showed no significant retention of sodium or potassium, although a significant increase in nitrogen excretion occurred. The patient gained 2½ pounds on the day following the infusion, the weight returning to normal on the subsequent day. Following trauma, such as surgical operations or fractures, no change in excretion of aldosterone occurred immediately after the damage. In 4 patients studied, a later rise in the output of aldosterone was observed which was maintained for several days. Electrolyte balance studies showed no retention of sodium. The excretion of sodium-retaining hormone in patients with rheumatoid arthritis usually fell within the normal range.

44. Effect of Aldosterone and Other Steroids on Renal Functions and Water Intoxication.

A. A. Renzi, Ph.D., Martha Renzi, M.A., J. J. Chart, Ph.D.* and *Robert Gaunt, Ph.D.** Research Department, Ciba Pharmaceutical Products, Inc., Summit, N.J.

The effects of aldosterone and other corticoids on the clearance of exogenous creatinine and para-aminohippurate and on the response to excess water were studied in adrenalectomized rats. Since the tests were stressful to adrenalectomized animals, the effectiveness of the steroids depended upon their "anti-stress," as well as renal effects. The steroids tested elevated the depressed Ccr and Cpah characteristics of the untreated adrenalectomized rat. There were no clearly established differences in potency between aldosterone and hydrocortisone. Cortisone was questionably weaker and 11-desoxycorticosterone definitely so. The M.E.D. of aldosterone was in the order of 12–25 μg. per 180-Gm. rat. Water excretion was increased by all treatments. The amount of aldosterone required to elevate clearance values was more than 200 times that needed to depress sodium excretion. In the water intoxication experiments, aldosterone, hydrocortisone and corticosterone were of about equal potency and at least 20 times as potent as 11-desoxycorticosterone. Aldosterone was active in doses of 62μg. or less per 180-Gm. rat. The biologic activities of aldosterone fall into three groups: (a) it is the most potent natural steroid in affecting sodium and potassium metabolism; (b) in the tests reported here and in the cold-stress test, its activity approximates that of hydrocortisone and cortisone; and (c) in classic glucocorticoid tests it has 1/3 the potency of cortisone.

45. Studies on Aldosterone in Man.

Robert H. Sheppard, M.D., William I. Morse, M.D., Albert E. Renold, M.D. and *George W. Thorn, M.D.** Department of Medicine, Harvard Medical School and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

The effects of aldosterone were studied in normal and Addisonian patients maintained in metabolic balance. As much as 1 mg. daily was administered for as long a period as one week. The hormonal activity was greatest when the compound was administered in a

continuous intravenous drip or intramuscularly in divided doses. Persistent, marked sodium retention resulted, and in an Addisonian patient treated for seven days there was a gain of 3.5 Kg. in weight and development of ankle edema. Although the effects on the urinary Na/K ratio were striking, the actual potassium changes were not great. A definite but slight eosinophil fall occurred in most experiments. Urinary glucose excretion and tolerance for intravenously administered glucose and fructose were not altered. Urinary nitrogen, uric acid and phosphate excretion remained relatively constant. Skin pigmentation, as measured by reflectance spectrophotometry, showed no lightening after 1 mg. was administered parenterally in divided doses daily for seven days. When the same dose was given to a normal subject for four days, no decrease in urinary steroid excretion, *i.e.*, no evidence of pituitary-adrenal suppression, was observed. In contrast, in the same subject, definite inhibition was obtained with an equal dose of 9-alpha-fluorohydrocortisone.

SATURDAY, JUNE 4, 1955
9:00 A.M. ADRENALS

CHAIRMEN: G. W. THORN AND J. W. JAILER

46. Exchangeable Body Sodium and Potassium Measurements in Hyper- and Hypoadrenocorticism.

Walter L. Arons, M.D., Bernardo Nusimovich, M.D.* and *Robert L. Vanderlinde, M.D.* Department of Medicine, Harvard Medical School and Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

Exchangeable body sodium (Na*e*) and potassium (K*e*) measurements have been made simultaneously (method of Arons, *et al.*; *J. Clin. Invest.* **33**: 1001, 1954) in 6 patients with Cushing's syndrome, 2 masculinized females, 1 patient with severe untreated Addison's disease and 5 Addisonian patients under treatment. Measurements were carried out on the Cushing's patients prior to, and three and twelve months following adrenalectomy. Before treatment these patients had relatively normal Na*e*'s and low K*e*'s, resulting in an abnormally high Na*e*/K*e* body ratio of 1.0 to 1.6 (normal = .84 for males, .94 for females). In Cushing's syndrome, up to twelve months was necessary for body electrolyte values to be restored to normal. A masculinized female with the adrenogenital syndrome had a male Na*e*/K*e* ratio (.74), whereas a hirsute female (Stein-Leventhal syndrome) had normal body electrolytes. The patient with untreated Addison's disease near crisis had *normal* Na*e* and K*e* values and a slightly elevated Na*e* and K*e* ratio. Body electrolyte values in 5 treated Addisonians were found to be within normal limits.

47. The Excretion of Pregnane-3α,17α,21-triol-20-one (Tetrahydro S) in Normal and Pathologic Urine.

J. C. Touchstone, Ph.D., H. Bulaschenko, M.S., E. M. Richardson, Ph.D. and *F. C. Dohan, M.D.** Endocrine Section of the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Philadelphia, Pa.

The urinary alpha-ketolic steroids were extracted with chloroform after glucuronidase and acid hydrolysis and separated by paper chromatography. Tetahydro S and cortisone do not separate well in the toluene-propylene glycol system but do in the benzene-formamide system. In most instances, identification included running rates as the free alcohol and the acetate; phenylhydrazine reaction; spectroscopy in concentrated sulfuric

acid; and in 4 instances infrared spectrometry (courtesy of Dr. Thomas F. Gallagher). The approximate values (μg. per day) for tetrahydro S extracted from the urine were: (a) "normal pool"—20, (b) 1 man and 2 women with hypertension—from undetectable to 70, (c) a woman with diabetic retinopathy—undetectable, (d) 2 men without adrenal disease, receiving corticotropin—250 and 450, (e) a man with Cushing's syndrome without adrenal tumor—1,700, (f) a woman with Cushing's syndrome with adrenal cancer—15,000, (g) a man with feminizing adrenal cancer—3,500, and (h) a woman with virilizing adrenal cancer—12,400. In the last case, a substance partially identified as S was found but was not detectable in the others, although metabolic studies indicated the chief source of tetrahydro S to be substance S.

48. Response of Plasma 17-Hydroxycorticosteroid Levels to ACTH in Adrenocortical Disease and in Pregnancy.

Eleanor Z. Wallace, M.D., Nicholas P. Christy, M.D. and *Joseph W. Jailer, M.D.** Departments of Medicine and of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons and Presbyterian Hospital, New York, N. Y.

Normal individuals respond to a standardized intravenous ACTH infusion with an elevation of the plasma 17-hydroxycorticosteroid level to 35–54 micrograms per 100 ml. (control 4–23), as measured by the Silber-Porter technic. Adrenocortical response to ACTH was studied in six groups of patients. Four such groups demonstrated subnormal rises: 1) In 8 Addisonians no rise in plasma 17-hydroxycorticosteroids occurred. 2) In 7 patients with hypopituitarism, post-ACTH levels ranged from only 6 to 21 micrograms per 100 ml. 3) In 5 of 6 patients with congenital adrenal hyperplasia, subnormal rises were observed. 4) In patients with rheumatoid arthritis treated with metacortandracin, there was partial to complete suppression of the plasma 17-hydroxycorticosteroid response to ACTH. Two groups of patients showed exaggerated rises in plasma 17-hydroxycorticosteroid levels after ACTH as follows: 1) In 3 patients with Cushing's syndrome due to bilateral adrenal hyperplasia, excessive corticosteroid increases occurred even after removal of one hyperplastic adrenal, but after subtotal bilateral adrenalectomy the corticosteroid rise was subnormal. One patient with adrenal carcinoma failed to show this hyper-responsiveness. 2) Normal pregnant women in the third trimester showed corticosteroid hyper-response comparable to that found in adrenal hyperplasia, in contrast to the complete lack of response encountered in a pregnant Addisonian patient.

49. Studies of Conjugates of C-21 Steroids in Human Plasma.

Alfred M. Bongiovanni, M.D. and *Walter R. Eberlein, M.D.** Pediatric Endocrine Laboratory, Children's Hospital, University of Pennsylvania, Philadelphia, Pa.

Methods were devised for the detection of corticoid conjugates and presteroids in human plasma. A 10-ml. sample of human plasma was added to 25 ml. of 95 per cent ethanol with stirring, allowed to stand for thirty minutes, and filtered. The filtrate was evaporated at 40°C. with air and the remaining 2–3 ml. was diluted with 10 ml. of water and extracted three times with 10 ml. of methylene chloride. The aqueous residue was incubated with acetate buffer (pH 4.5) and 3,000 units of glucuronidase at 37°C. for forty-eight hours. Following incubation, this was extracted in the same manner. Both fractions were then assayed for "Porter-Silber" chromogens by chromatography on Florisil according to the method of Nelson and Samuels (*J. Clin. Endocrinol. & Metab.*

12: 519, 1952). This system had several advantages. It permitted the determination of corticoid conjugates, troublesome emulsions were avoided and the quantities of glucuronidase were reduced. Aliquots of both free and conjugated fractions, following the administration of known steroids, were identified by reactions with phenylhydrazine hydrochloride, periodate oxidation, paper chromatography in various systems, melting points and in some instances by infrared spectroscopy. The material extracted as "conjugates" generally represented the reduced components of the administered precursors. Following administration there was a rapid rise of the free steroid fraction followed by a slower rise of the conjugated fraction.

50. Metabolic and Endocrinologic Consequences of Abrupt Cessation of Long-Term Cortisone Therapy in Rheumatoid Arthritis.

Evan Calkins, M.D., Walter Bauer, M.D.,* Lewis L. Engel, Ph.D.** and *Priscilla Carter, M.A.* Massachusetts General Hospital and Harvard Medical School, Boston, Mass.

The effects of the withdrawal of cortisone were examined in 3 patients with rheumatoid arthritis maintained on cortisone for two or more years. Studies included electrolyte and nitrogen balances in all 3 patients, and daily determinations of urinary corticosteroid excretion in 1 (J.L.). For the three days following abrupt cessation of cortisone therapy the patients displayed symptoms and metabolic changes suggestive of adrenal insufficiency. In J. L. there was a concomitant fall in the urinary excretion of tetrahydrocortisone and tetrahydrocortisol to less than 5 per cent of the control levels. On the fourth and fifth days, these symptoms subsided, the patients entered a phase of sodium retention and worsening of the arthritis, and J. L. exhibited a substantial increase in tetrahydrocortisone and tetrahydrocortisol excretion. In addition, two less polar reducing substances were observed in the corticosteroid chromatograms. These substances had been seen previously when the patient was receiving ACTH superimposed upon cortisone. They were seen again on the ninth day when ACTH alone was administered. The evidence suggests that the effects of endogenous ACTH secretion became manifest on the fourth day following cessation of cortisone therapy.

51. Studies of the Kinetics of the Adrenocortical Response to Corticotropin.

V. Di Raimondo, M.D. and *D. Island, B.S.* (introduced by P. H. Forsham, M.D.). Metabolic Unit for Research in Arthritis and Allied Diseases, University of California School of Medicine, San Francisco, Calif.

Utilizing hourly changes in urinary 17-hydroxycorticoids, the kinetics of adrenal activation have been investigated. A close quantitative and temporal correlation between urinary 17-hydroxycorticoids and blood free 17-hydroxycorticoids has been demonstrated. For doses of ACTH of 1 USP unit and above, given in 8-hour intravenous infusions, there is a maximal acceleration of steroidogenesis. However, for doses of .5 and .25 USP units the acceleration is submaximal, and .25 USP units produced no response. From these studies one would estimate a daily basal output of ACTH biologically equivalent to less than 1 USP unit.

A diurnal variation in adrenocortical response to exogenous ACTH has been demonstrated, which parallels the spontaneous diurnal changes in adrenal activity. Li's sheep alpha-corticotropin has been characterized in man. Using doses of equal adrenal ascorbic-acid depleting capacity, its steroidogenic action was compared with that of a highly purified hog ACTH. Whereas the acceleration of steroidogenesis during the infusion of

ACTH was the same, the duration of enhanced steroidogenesis was found to be greater for the hog preparation. Species differences, or the absence of another steroidogenically active factor in the pure alpha-corticotropin, may be responsible for this dissimilarity.

52. Species Variations in the Response of Serum Proteins, Lipids and Lipoproteins to Cortisone.

Elaine T. Bossak, M.D., Chun-I. Wang, M.D. and *David Adlersberg, M.D.* Mount Sinai Hospital, New York, N. Y.

Previous studies demonstrated elevation of the level of serum (plasma) lipid fractions in man and rabbit under the influence of large doses of cortisone. More recently, we have studied the influence of cortisone on the level of serum (plasma) proteins and lipoproteins by means of paper electrophoresis and chemical estimation of lipid partition. Amidoschwarz 10B was used for protein staining and Oil Red O for lipids, with quantitative estimation by densitometry. A selective elevation of plasma alpha-2-globulin concentration to twice normal, and a drop in beta-globulin were noted in dogs receiving cortisone with or without cholesterol supplements, but not in man or the rabbit. Combination of cortisone injections and cholesterol feeding in dogs resulted in elevation of the levels of plasma total and esterified cholesterol, phospholipids, neutral fat and total lipid to 2 or $2\frac{1}{2}$ times normal. Marked alteration of the plasma lipoprotein pattern was observed. Beta-lipoprotein plus 0 fraction (lipid adsorbed at the origin) accounted for more than half of the stainable lipid. The combination of cortisone administration and cholesterol supplements in the rabbit resulted in marked enhancement of lipid elevations induced by cholesterol feeding alone. Total cholesterol concentration rose to levels 40 times normal, and the levels of the other lipid fractions were elevated to about 10 times normal. The lipoprotein profile was completely distorted and characterized by almost total disappearance of alpha-lipoprotein and extreme elevations of the beta plus 0 fraction. These results suggest that adrenal steroids affect to a greater extent lipid metabolism in man and rabbit, and protein metabolism in the dog.

53. The Prophylactic Use of Cortisone for Surgery of the Pituitary.

Philip Troen, M.D. and *Edward H. Rynearson, M.D.** Mayo Foundation and Mayo Clinic, Rochester, Minn.

To determine the value of the preoperative use of cortisone in operations on, or about the pituitary, 163 such surgical procedures from 1949 through 1953 were reviewed. There were 127 pituitary adenomas, 25 craniopharyngiomas and 11 miscellaneous lesions. Sixty patients did not receive cortisone preoperatively; 93 received it preoperatively. Classification as to the degree of preoperative anterior-pituitary insufficiency revealed: none (or minimal), 94; mild, 37; marked, 22. The patients' records were analyzed for complications (beginning within the first five postoperative days) which could be ascribed to anterior-pituitary failure. There were 10 endocrine complications consisting of acute adrenocortical failure or the development of hypotension or drowsiness and stupor responding to adrenal replacement therapy. There was a significantly greater incidence of postoperative endocrine complications in patients with marked preoperative pituitary insufficiency. In patients with marked insufficiency, the preoperative administration of cortisone significantly decreased the incidence of postoperative endocrine complications. There were 16 deaths with no relation to preoperative insufficiency, and all but 1 appeared to be "nonendocrine." The mortality rate was 17 per cent among those not receiving cortisone and 6 per cent among those receiving cortisone preoperatively.

54. The Effect of Estrogen and Androgen on Calcium Metabolism during Cortisone Administration.

Philip H. Henneman, M.D., John W. Irwin, M.D., David M. K. Wang, M.D.* and *Walter S. Burrage, M.D.* Medical Service of the Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Boston, Mass.

It appears that progressive osteoporosis will be the most common serious consequence of prolonged high dosage cortisone or hydrocortisone therapy. The possible blockade of this anti-anabolic effect of hypercortisonism by anabolic hormones is thus of therapeutic and theoretical interest. Despite certain limitations, the 24-hour urinary calcium excretion provides a convenient index of the progression or regression of osteoporosis in such patients. Twenty-eight patients with chronic intractable asthma of unknown etiology received prolonged cortisone suppression therapy for asthma. Repeated measurement of daily urinary calcium excretion indicated that the majority of these patients had hypercalcuria; and hence, presumably, osteoporosis was developing. Urinary calcium excretion was markedly reduced during brief cessation of cortisone therapy. Large doses of estrogen in the females, or androgen in the males, reduced to normal the urinary calcium excretion of the patients with hypercalcuria, but were without effect in the patients with normal urinary calcium excretion. It would appear from the urinary calcium excretion data that estrogen and androgen in large doses may block the progression of the osteoporosis of induced hypercortisonism.

55. Addison's Disease with Normal Basal Secretion of 17-Hydroxycorticoids.

Najib Abu Haydar, M.D., John C. Laidlaw, M.D., William J. Reddy, A.B.** and *George W. Thorn, M.D.** Department of Medicine, Harvard Medical School and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

The signs and symptoms of Addison's disease are well recognized and are ascribed to complete adrenocortical insufficiency. The occurrence of partial adrenocortical insufficiency, however, has been a subject of controversy. Recently a group of patients has been observed, showing one or more of the signs and symptoms of classical Addison's disease while excreting normal quantities of 17-hydroxycorticoids in the urine. The most prominent sign was hyperpigmentation. While enjoying a state of well-being under normal conditions, these patients presented evidence of adrenocortical insufficiency under stress. In contrast to the complete Addisonian patient, these patients were found to excrete normal amounts of 17-hydroxycorticoids in the urine, but their corticoid excretion failed to rise upon maximal stimulation with intravenous ACTH for two or more days. Chromatographic separation showed tetrahydrocortisone to be the major urinary corticoid in quantities comparable to those obtained by the Reddy method (*Metabolism* **3**: 485, 1954). These results suggest that these patients possess adrenal tissue which is able to supply their basal needs but is unable to meet the hormonal demands of superimposed stress. Diagnosis is established by a failure to respond to repeated administration of ACTH intravenously, and not by basal steroid excretion values.

56. Effects of Cortisone and Insulin on Urinary Excretion of Radioactive Vitamin B_{12} in Rats.

*Joseph Meites, Ph.D.** and *Y. S. L. Feng, Ph.D.* Department of Physiology and Pharmacology, Michigan State College, East Lansing, Mich.

In rats fed a vitamin B_{12}-deficient diet, injections of 2 mg. of cortisone daily doubled, and 4 mg. of cortisone daily tripled the urinary excretion of tracer doses of radioactive

vitamin B_{12}. On a diet just adequate in vitamin B_{12}, 2 mg. of cortisone daily produced no change, but 4 mg. increased urinary vitamin B_{12} losses about 50 per cent. Injections of insulin markedly reduced the urinary losses of vitamin B_{12} in normal, alloxan-diabetic and cortisone-injected rats, whether or not they were fed vitamin B_{12}. In the cortisone-injected rats, insulin was only about 1/3 as effective in this respect in the vitamin B_{12}-deficient as in the vitamin B_{12}-adequate rats. These results are in agreement with the view that vitamin B_{12} is essential for normal carbohydrate metabolism (Chow, 1952–54). The increased urinary excretion of radioactive vitamin B_{12} in the cortisone-treated rats is believed to reflect the increased insulin resistance and reduced utilization of carbohydrate observed in these animals, whereas the reduced urinary losses of the vitamin in the insulin-injected rats is believed to reflect the increase noted in glucose utilization.

57. Aminopyrine Inhibition of the Plasma 17-Hydroxycorticosteroid Increase Produced by the Pyrogenic Reaction.

Nicholas P. Christy, M.D., Anthony Donn, M.D. and *Eleanor Z. Wallace, M.D.* (introduced by Joseph W. Jailer, M.D.). Departments of Medicine, Obstetrics and Gynecology, and the Institute of Ophthalmology, Columbia University, College of Physicians and Surgeons and Presbyterian Hospital, New York, N. Y.

In 6 patients with various ophthalmologic disorders intravenous typhoid vaccine (5–15 million killed organisms) caused a sharp rise in plasma 17-hydroxycorticosteroid levels as measured by the Silber-Porter technic. Peak corticosteroid increases coincided closely with the maximum temperature rise, and were comparable to levels attained after standard intravenous ACTH tests (36–59 micrograms per 100 ml.). An attempt was made to determine whether this adrenocortical stimulation depended solely on the febrile response to typhoid, or could be produced in the absence of fever. Since it is known that the administration of aminopyrine inhibits the febrile response but does not prevent certain hemodynamic effects characteristic of the pyrogenic reaction (Bradley, S. E., *et al.*), the drug was given to 3 patients over the 24-hour period prior to the typhoid dosage. The febrile reaction was suppressed. No rise in plasma 17-hydroxycorticosteroids occurred, although the drug failed to abolish other systemic effects of vaccine (weakness, nausea). It was concluded that the action of aminopyrine on the central nervous system, by blocking the pyrogenic effect of the typhoid vaccine, prevented the stimulus to ACTH-release and hence to adrenocortical activation.

2:00 P.M. JOINT SESSION WITH AMERICAN DIABETES ASSOCIATION

CHAIRMEN: H. B. MULHOLLAND AND A. T. KENYON

DIABETES

58. Hypophysectomy in Severe Juvenile Diabetes.

Laurance W. Kinsell, M.D., Lester Lawrence, M.D.* and *Robert D. Weyland, M.D.* Departments of Medicine and Neurosurgery, Samuel Merritt Hospital, and Institute for Metabolic Research of Highland Alameda County Hospital, Oakland, Calif.

Seven hypophysectomies have been performed in 6 juvenile diabetics, with no operative mortality. It was necessary to re-operate on 1 patient because hemorrhage prevented complete removal of the gland. The observations were: 1. All patients had major eye and kidney disease. The last 4 had sufficient renal reserve for maintenance of a normal blood urea nitrogen level with an intake of 60 grams of protein daily. 2. All had a

major decrease in insulin requirement, the average dose on a relatively high carbohydrate intake being less than 15 units of insulin, instead of over 60 units. 3. Hypertension, when present, disappeared. 4. Albuminuria diminished significantly, but did not disappear completely. Tolerance for dietary protein increased. 5. Pneumonitis in 1 patient increased albumin excretion, and evidence of renal and retinal damage. The cortisone requirement increased from 10 mg. to 100 mg. daily. 5. Erythropoiesis improved in 2 of the patients. The maintenance medication used was insulin, thyroid, cortisone, testosterone and, in females, estrogen. There was suggestive evidence that the amount of testosterone bore a critical relationship to the efficiency of erythropoiesis.

59. The Metabolism of C^{14}-Labeled Glucose in Normal and Diabetic Subjects.

Max Miller, M.D., R. A. Shipley, M.D., Nome Baker, Ph.D., W. W. Shreeve, Ph.D., M.D.* and *J. W. Craig, M.D.* Departments of Medicine and Biochemistry, School of Medicine, Western Reserve University; Lakeside Hospital; and the Radioisotope Unit, Veterans Administration Hospital, Cleveland, Ohio.

A dose of 100 μc. of uniformly labeled C^{14} glucose was injected intravenously into 4 normal and 8 diabetic subjects deprived of insulin. During the following six hours, specific activities of blood glucose and of expired CO_2 were determined serially. The urinary excretion of labeled glucose in the diabetic subjects was also analyzed. For the normal and diabetic subjects the volume of distribution of glucose was 17 per cent and 23 per cent, respectively, of body weight. The difference between the diabetics and normals was not statistically significant. Normal subjects gave a mean value of 22 per cent (range 17 to 29 per cent) of CO_2 derived from glucose. In 5 "stable" diabetic subjects, the mean value was 20 per cent (range, 16 to 31 per cent). In 3 "labile" diabetics in whom there was prompt development of progressive ketosis upon withdrawal of insulin, the average was 10 per cent (range, 9 to 12 per cent). Calculations of mg.-glucose/Kg./hr. oxidized averaged 38 for the normal group, and 49 and 18 for the two respective diabetic groups. Total turnover rate for normal subjects was 62 mg./Kg./hr., 86 for the stable diabetics, and 120 for the labile diabetics, suggesting that glucose production by the liver is accelerated in the diabetic subjects. The percentage of glucose disappearing from the blood and unaccounted for either by oxidation or urinary excretion in the diabetics was approximately 5 per cent as compared to 39 per cent in normal subjects, indicating a *decreased formation* of glycogen and fat in the diabetic.

60. Interactions of Growth Hormone, Cortisone, and Triiodothyronine in Experimental Diabetes in the Rat.

J. A. Owens, Jr., M.D., M. D. Bogdonoff, M.D. and *F. L. Engel, M.D.** Duke University, Durham, N. C.

Previous investigation in this laboratory has shown that combined daily injection of cortisone, 2.5 mg., and growth hormone, 2 mg./100 Gm. body weight, into intact, tube-fed produces a significant hyperglycemia (353 ± 97 mg./100 ml.), if two-hour post-feeding blood sugars for treatment days 4 and 5 are pooled. Either hormone alone was subdiabetogenic at these dosages. Utilizing this method, the relation of thyroid to these hormonal effects was studied, using triiodothyronine, 5 μg./100 Gm. body weight daily. Blood sugar elevations above pre-treatment levels were (mean $\pm$S.E.): triiodothyronine alone, $+66 \pm 3.5$ mg. per 100 ml.; triiodothyronine plus growth hormone, $+37 \pm 9.5$ mg. per 100 ml.; and triiodothyronine plus cortisone, $+75 \pm 2.0$ mg. per 100 ml. Triiodothyronine alone produced definite diabetes; triiodothyronine plus growth hor-

mone, borderline diabetes. Both cortisone and growth hormone alleviated the thyrotoxic severity and mortality, cortisone causing a greater, and growth hormone a lesser weight loss than triiodothyronine alone. Similar investigations with varying triiodothyronine dosages but a constant dose of cortisone or growth hormone revealed a definite relationship between loss of weight and increase in blood sugar level. Increasing triiodothyronine dosages effected a greater increase in blood sugar concentration per gram of weight lost. Triiodothyronine (2.5 μg./100 Gm.) plus growth hormone and cortisone abolished the diabetes ordinarily resulting from the latter two hormones in combination (blood sugar levels, 190 ± 5.8 mg./100 ml. versus 353 ± 97 mg./100 ml.).

61. Effects of Glucose Levels on Lipogenesis and Oxidation in Rat Liver and Adipose Tissue.

S. W. Milstein and *F. X. Hausberger, M.D.** Jefferson Medical College, Philadelphia, Pa.

The *in vitro* effect of glucose concentrations ranging from 50 to 1600 mg. per 100 ml., on the incorporation of uniformly labeled C^{14}-glucose into fatty acids and CO_2 was investigated. In normal liver, lipogenesis did not occur below 400 mg. per 100 ml. but increased linearly with higher concentrations. Adipose tissue had an active C^{14} incorporation into fatty acids at 50 mg. per 100 ml., higher concentrations evoking little increase. Diabetic tissues had negligible fat synthesis at all glucose levels. Insulin treatment of normal rats enabled the liver to commence lipogenesis at 50 mg. per 100 ml. and respond to higher concentrations with greatly increased rates. Adipose tissue from such animals showed an enhanced fat synthesis far exceeding that in the liver at low concentrations, but approaching it at higher concentrations. The rate of glucose oxidation in diabetic liver slices at 400 mg. per 100 ml. was equal to that observed in normal liver at 100, both concentrations approximating, respectively, the prevailing glycemic conditions. In both cases, rates were clearly dependent on glucose levels, whereas in corresponding adipose tissues, plateau values were seen throughout the range studied, with a slight depression in the diabetic tissues. Insulin treatment increased the oxidation rates in both tissues without distorting their characteristic responses to glucose concentration.

62. The Distribution and Degradation of I^{131}-Labeled Insulin in Normal Subjects and in Diabetics.

*George W. Welsh, Elaine D. Henley, Robert H. Williams, M.D.** and *Neil J. Elgee.* University of Washington School of Medicine, Seattle, Wash.

Since diabetes mellitus may be due to factors other than pancreatic insulin insufficiency, inactivation of insulin was studied. I^{131}-insulin in tracer amounts was given intravenously to 40 diabetics, and to 15 nondiabetic controls. All subjects were fasting, and therapeutic insulin was withheld for 24–96 hours. Glucose was given orally, in proportion to the amount of labeled insulin injected. Plasma and urine samples were precipitated with trichloracetic acid. The radioactivity in the precipitate (presumably insulin-bound) and supernatant fraction (degradation products) was measured. Plasma samples from some subjects were incubated *in vitro* with I^{131}-insulin and the percentage of degradation determined. In the control series the total plasma precipitable radioactivity showed a rapid decrease from the initial theoretical 100 per cent content to an average of 20 per cent of the dose in fifteen minutes, and 7.5 per cent in sixty minutes. Diabetics maintained markedly higher levels of plasma precipitable radioactivity—up to

80 per cent in sixty minutes. Supernatant radioactivity increased progressively in controls, but remained low in diabetics. Urinary radioactivity in controls averaged 10 per cent of the dose in sixty minutes, whereas diabetics generally excreted much less. Epithyroid counts were higher, in controls; no differences were recorded over other organ areas. *In vitro*, there was less degradation of I^{131}-insulin in diabetic than in normal plasma, over a 3-hour period. These studies indicate that the disappearance and extravascular dispersion of I^{131}-insulin from the plasma in the diabetic is retarded, and that degradation is delayed.

READ BY TITLE

THYROID AND PARATHYROIDS

63. Measurement of Salivary and Gastric Secretion of I^{131} in the Rat.

Josiah Brown, M.D. (introduced by E. B. Astwood, M.D.). New England Center Hospital, and Tufts Medical School, Boston, Mass.

To measure iodide secretion by the salivary and gastric glands of the rat, a catheter was tied into the stomach through the duodenum under ether anesthesia. After the animal awakened, a dose 5–10 μc. of I^{131} was injected subcutaneously, thyroidal protein binding being inhibited by propylthiouracil. Combined gastric and salivary secretions were collected at intervals during eight to twelve hours, using an external rubber balloon and gastric lavage. Blood specimens (0.3 ml.) were taken at 2-hour to 3-hour intervals. Salivary plus gastric secretion of I^{131} approached a calculated total of 70.7 per cent of the administered dose, with a clearance value of 0.41 cc./min., half of the total being collected in the first 3.8 hours. When the salivary glands were previously removed the total was 57.4 per cent, the clearance .29 cc./min., and half-life 4.0 hours. It is estimated that the salivary glands contributed about one fifth of the I^{131} in the combined salivary and gastric sectetions. Subcutaneous administration of 10 mg. of potassium thiocyanate one hour before the I^{131} resulted in a maximum of 55.6 per cent, the gastric plus salivary clearance was reduced about one-third, and the half-life was prolonged. Treatment with 10 μg. of L-thyroxine daily for three days did not significantly change the rate of secretion of I^{131} by salivary and gastric glands.

64. Depression of Serum Protein-Bound Iodine Levels in Man With Dinitrophenol.

C. William Castor, M.D. and *William H. Beierwaltes, M.D.* (introduced by Gardner M. Riley, Ph.D.). University of Michigan, Ann Arbor, Mich.

In 1950 Wolff described significant lowering of the serum protein-bound iodine level in rats made hypermetabolic with 2,4-dinitrophenol. Isotopic and histologic studies have failed to clarify the mechanism by which the serum protein-bound iodine level is depressed. The present study showed in 9 human beings that the same phenomenon occurred in man when 450 mg. of 2,4-dinitrophenol was ingested over a two-day interval in divided doses. Serum PBI levels fell approximately 23 per cent from control levels when dinitrophenol was administered. The difference between the means of the control and experimental PBI values in these 9 individuals was significant beyond the 1 per cent level on statistical analysis. Preliminary data indicated that increased urinary and fecal excretion of PBI was not an important factor. Further, it appeared that increased adrenocortical activity did not occur following administration of 2,4-dinitrophenol. It is possible that pituitary thyrotropic activity is depressed by the calorigenic effect of 2,4-dinitrophenol.

65. Effect of Continuous Infusion of TSH in Guinea Pigs.

D. A. DeBias, F. W. Sunderman, M.D., A. Cantarow, M.D.** and *K. E. Paschkis, M.D.** Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa.

Experiments were undertaken to study whether excessive stimulation of a gland causes functional exhaustion and/or cell damage. TSH (Parke-Davis) was administered by continuous intravenous infusion to unanesthetized guinea pigs for twelve to ninety-six hours. The dose of TSH was adjusted so that in one series all animals received approximately the same dose, and only the duration of administration varied; in a second series the dose per unit time was constant, and consequently the animals received proportionally more TSH with increased duration of the experiment. Control animals were simultaneously infused with 0.85 per cent NaCl solution. The following determinations were made at the end of the experiments: thyroid weight, thyroid histologic pattern, serum protein-bound iodine level, and thyroidal I^{131} uptake twenty-four hours after injection of a tracer dose of 8 microcuries. The changes of thyroid weight, of the histologic picture of the thyroid tissue and of the I^{131} uptake (calculated per mg. of thyroid tissue) indicated increasing stimulation with increasing dose and length of administration. In no instance was cell necrosis or hemorrhage observed, such as is seen in adrenal glands similarly treated with ACTH (Ingle, D. J.; *Ann. Int. Med.* **35:** 652:– 1951). The PBI concentration increased, but with more intense stimulation (increased dose and duration) the values declined below those of the controls.

66. Method for Identifying Thyroxine-Binding Globulin in Protein Mixtures: Localization of TBG in Cohn Fractions.

Norbert Freinkel, M.D., James T. Dowling. M.D.* and *Sidney H. Ingbar, M.D.** Thorndike Memorial Laboratory, Second and Fourth Medical Services, Boston City Hospital, and Harvard Medical School, Boston, Mass.

To assess the role of the thyroxine-binding alpha-globulin (TBG) in transcellular exchanges of thyroxine, a concentrated source of TBG was sought. Outdated blood-bank and fresh A.C.D. plasmas were fractionated according to Method 6 of Cohn (*J. Am. Chem. Soc.* **68:** 459, 1946; **71:** 1223, 1949). Cohn fractions (3 Gm. per cent) were added to normal sera which had been supplemented with stable and I^{131}-thyroxine so that bound radioactivity was distributed between TBG and albumin. It was assumed that Cohn fractions containing TBG in excess of the normal TBG/albumin ratio should enhance the apparent thyroxine binding of the alpha globulin and diminish the "spill-over" onto albumin. Control and protein-enriched sera were subjected to zone electrophoresis at pH 8.6 and 4.5. At both pH's only subfractions of IV-4 altered the binding partition of thyroxine. IV-6 exhibited the most activity and doubled the radioactivity which migrated with the TBG while correspondingly reducing the albumin-bound moiety. Preliminary observations suggest that enrichment of serum with IV-6 does not enhance the cellular penetration of thyroxine *in vitro*.

67. Goiter in Peru, Chile and the Argentine Republic.

*Isidor Greenwald, Ph.D.** New York University College of Medicine, New York, N. Y.

Peru. Goiter was not endemic in any part of the Inca Empire at the Conquest. A century later Calancha wrote "only in the hands of Cuzco and Chuquisaca is a water

which causes people to develop swellings in the throat called "cotos." Similarly restricted occurrences were reported by two others after another century. General endemicity is rather recent. **Chile.** Goiter was first reported in 1814. Some years later, there were several statements that it first became prevalent around Santiago about 1820. In that year, it was claimed that it had been seen nowhere else in Chile in the course of a thousand miles. It is now widely distributed. **Argentine Republic.** In 1658, nearly all the women of Santiago del Estero had goiter but there was no mention of its presence in places (Jujuy, Salta) in which goiter later became extremely prevalent. By 1870, its incidence in certain places was diminishing. This later became more general and, by 1914, was so marked that no separate enumeration was made in the census of that year. The number of deaf-mutes also decreased and, apparently, is still diminishing; all without benefit of iodine.

68. A Study of the Effect of Stable Iodide and Thyrotropin on Thyroid Secretion in Hyperthyroidism.

*Monte A. Greer, M.D.** and *Leslie J. DeGroot, M.D.* National Cancer Institute, Besthesda, Md.

The thyroid secretion rate was studied in the following subjects: 13 thyrotoxic, 14 euthyroid, and 1 post-radiothyroidectomy. A dose of 30 to 60 mg. of methimazole was given every eight hours beginning twenty-four hours after a 100-μc. tracer dose of I^{131}, to block thyroidal reaccumulation of I^{131}. Counts were made over the thyroid region daily and corrected for physical decay. The average half-time of the decline in thyroidal radioactivity (secretion rate) in the thyrotoxic patients was 4.9 days (range 1.5–8.0). Nine patients then received 300 mg. of NaI daily. In all there was a significant slowing of the secretion rate: average half-time, 110.9 days (range 5.5–532). Inhibition appeared in an average of 1.8 days and reached a maximum in 3.4 days. In 7 of 8 subjects who then received TSH during maximal iodide inhibition, the secretion rate was returned to approximately its pre-iodide level. Four thyrotoxic subjects received single doses of 25 to 300 mg. of NaI. All had a transient inhibition of the secretion rate, which was related to the level of blood iodide. Iodide had no effect upon secretion rate in non-thyrotoxic subjects or upon that induced by TSH in euthyroid subjects. It is suggested that iodide acts in thyrotoxicosis by inhibiting release of TSH, and that thyrotoxicosis in most cases is probably due to a dysfunction of suprathyroidal centers and not to primary hyperfunction of the thyroid gland.

69. Creatine-Creatinine Excretion in Cold-Exposed Rats.

*Seymour Katsh, Ph.D.** Biology Division, California Institute of Technology, Pasadena, Calif.

Thirty-two adult male albino rats were evenly divided into four groups and placed in metabolism cages. Groups I and II were maintained at laboratory temperatures for control, the former receiving a standard diet and the latter a diet containing 33 mg. and 6 mg. of betaine and glycocyamine, respectively, per 100 Gm. of body weight per day. Groups III and IV received the matching diets of I and II; after an equilibration period they were exposed to 4°C. for intervals to twenty-eight days. Twenty-four-hour urine samples were collected. Control-fed rats at laboratory temperature showed a steady average excretion of 12–14 mg. of creatine per 100 ml. of urine. Twenty-four-hour urine samples from control-fed rats after two, four, and twenty-six days of cold showed 34, 40, and 27 mg., respectively, of creatine per 100 ml. of urine. During the cold, creatinine excretion was reduced by 40–50 per cent. In the rats fed betaine and glycocyamine,

creatine excretion, which ranged between 80 and 100 mg. per 100 ml. of urine during each 24-hour period, was unaltered during cold. Creatinine excretion, however, was similar to that in the control-fed rats. Creatinuria has been related to thyroid stimulation. Histologically, the thyroids were intensely stimulated in the cold-exposed groups. It appears that feeding precursors did not alter the creatininemia or affect the thyroid response resulting from cold exposure.

70. Reliability of Scintiscanning Nodular Goiters in Judging the Presence or Absence of Carcinoma.

Philip C. Johnson, M.D. and *William H. Beierwaltes, M.D.* (introduced by Gardner M. Riley, Ph.D.). University of Michigan, Ann Arbor, Mich.

From November 1953 to December 1954 the nodular goiters of 208 patients were scanned with a scintillation tube, moved automatically, twenty-four hours after the administration of 300 microcuries of I^{131}. Nodules concentrating I^{131} more avidly than normal thyroid tissue were classified as "hot"; those concentrating the same as normal thyroid tissue as "warm"; and less than normal thyroid tissue as "cold." Of the patients so scanned, 55 per cent had cold nodules, 28 per cent had warm, and 17 per cent had hot nodules. Forty-four of the original 208 patients were subjected to thyroidectomy. Carcinoma was found histologically in 14 of 32 patients (31 per cent) with cold nodules. Four of these patients had multinodular goiters clinically. Carcinoma was diagnosed histologically in 1 out of the 10 warm nodules (10 per cent) but was not found in either of the 2 hot nodules. The carcinomatous warm nodule was a clinically solitary nodule. Correlations of these findings were made with other factors of possible prognostic significance, such as roentgen evidence of calcification within an adenoma, and duration of goiter. It is concluded that routine scintiscanning of nodular goiters is a definite aid aid to clinical judgment.

71. Relationship Between Thyrotropin Effects Upon Human Thyroid Hormone Release and Iodide Uptake.

R. P. Levy, M.D., L. W. Kelly, Jr., M.D.,* G. W. Cooper, M.R.A.C.P.* and *McK. Jefferies, M.D.** Western Reserve University, Cleveland, Ohio.

A single injection of TSH normally produces within twenty-four hours an increased thyroidal uptake of I^{131} and a rise in serum PBI concentration, reflecting an increased rate of iodide trapping and of hormone release, respectively. In a subject with normal thyroid function in whom further synthesis of hormone had been blocked (with methimazole), injection of TSH still produced a significant rise in PBI concentration within twenty-four hours, indicating a release of previously stored hormone. Conversely, normal subjects whose glands had been rested by thyroid administration for a sufficient period had a rise in thyroidal I^{131} uptake without change in the level of serum PBI, implying: 1) the presence of insufficient stored hormone to produce a rise in the PBI level, 2) inability to resume synthesis of hormone sufficiently rapidly to raise the concentration of PBI within twenty-four hours, and 3) that increased uptake of iodide can occur without measurable release of hormone from the gland. Furthermore, in certain hyperthyroid patients, TSH caused a rise in PBI concentration without significant change in I^{131} uptake, indicating that increased hormone release can occur without a rise in uptake. It therefore appears that the rise in the serum PBI level occurring within twenty-four hours of a single injection of TSH results primarily from release of stored hormone, and that TSH can influence iodide uptake and hormone release independently.

72. A Defect in the Function of the Rat Thyroid Induced by I^{131} Irradiation.

F. Maloof, M.D. and *B. Rapp* (introduced by J. B. Stanbury, M.D.) Massachusetts General Hospital, Boston, Mass.

Two hundred male Sprague-Dawley rats (125±15 Gm.) were given a low-iodine diet (0.1γ/Gm. of food) for fourteen days, and groups of these animals were then given single doses of 0, 5, 20, 100 and 200 μc, of I^{131} intraperitoneally. Forty-eight hours later, a regular diet (2.0γ/Gm. of food) was given. One month after radiation, animals which had received 5 to 200 μc. of I^{131} showed an increase in the height of the thyroid cells, the thyroid/serum (T/S) ratio, the thyroidal iodine space and the percentage of the thyroidal iodine (I^{127}) released each day. The level of serum PBI^{127} was low in the 100 μc. and 200-μc. groups; and the pituitaries showed marked evidence of increased TSH output in these animals. Four months after radiation, the T/S ratio and the thyroidal iodide space in the irradiated groups dropped to values 1/2 and 1/3 of those in the control group: eight and twelve months after radiation, these parameters were approximately equal in the 0 to 100-μc. groups. The low level of PBI^{127} persisted. In the 200-μc. group the thyroid weight and functions declined gradually to a level well below normal. The increased follicular cell height and the evidence for increased TSH output persisted. From these data, it appears that the thyroid mechanisms most sensitive to I^{131} irradiation are those that convert the concentrated iodide to thyroid hormone.

73. The Influence of Renal Factors on Iodide Kinetics.

Howard Rasmussen, M.D., Malcolm Powell, A. B. and *Betty Rapp* (introduced by J. B. Stanbury, M.D.). Massachusetts General Hospital, Boston, Mass.

Certain patients with cardiac or renal disease have been found to have a high 48-hour thyroidal uptake of radioiodine. A study was undertaken to define the effects of these disease states on iodide kinetics. The renal clearance rates (k_2) and thyroidal clearance rates (k_1) were calculated in two ways in most patients, utilizing the data obtained by direct counting over the thyroid and either the disappearance rate of I^{131} from the blood stream or renal excretion of I^{131}. The rate of production of PBI^{131} was measured in some. The patients were found to fall into two groups. In the first, the thyroidal clearance rate (k_1) was normal, and the renal clearance rate (k_2) low. This resulted in a high 48-hour uptake of I^{131} by the thyroid. The second group had low k_1 and low k_2 and consequently had either normal or low 48-hour I^{131} uptakes. There was a rough correlation between thyroidal clearance rates and the chronicity of the disease, the rates being lower the more chronic the disease state.

74. Pitfalls in the Diagnostic Use of Radioactive Iodine.

Solomon Silver, M.D., S. B. Yohalem, M.D.* and *R. A. Newburger, M.D.* Mount Sinai Hospital, New York, N. Y.

Two techniques involving tracer doses of I^{131} in the diagnosis of hyperthyroidism have received widespread, if uncritical, acceptance. Thyroidal uptake and serum protein-bound I^{131} levels at seventy-two hours after the tracer dose are the two commonly used. Data were collected on 113 patients, all of whom were euthyroid at the time of the I^{131} studies, and the euthyroid state was established by clinical signs, B.M.R., and serum PBI concentration. In each of these 113 patients either the thyroidal uptake was greater than 55 per cent or the PBI^{131} plasma levels were in excess of 0.3 per cent of the administered dose per liter of plasma—values which we accept as the upper limits of

normal. This group included patients cured of previous hyperthyroidism by surgery or I^{131}, nontoxic nodular or diffuse goiters, those in the recovery phase after antithyroid drugs, iodides, desiccated thyroid, or thyroiditis, and a considerable number in whom no overt cause for the discordant results could be determined. A decreased iodide pool and a decreased intrathyroidal hormonal iodine pool in the mechanism of these "false" results is suggested. Awareness of the possibility of these "false hyperthyroid" values will prevent unnecessary treatment of euthyroid patients and further treatment of cured hyperthyroid patients, with resultant myxedema.

75. Action of Antithyroid Substances on the *In Vivo* Conversion of Diiodotyrosine to Thyroxine.

D. Ward Slingerland, M.D. (introduced by E. B. Astwood, M.D.). New England Center Hospital and Tufts Medical School, Boston, Mass.

Certain antithyroid substances have been administered to rats to determine the effect on the conversion of mono- and diiodotyrosine to thyroxine. Control rats were sacrificed three to six hours after the intraperitoneal injection of 50 μc. of carrier-free I^{131}, at which time test animals were given various antithyroid substances for twenty-four to forty-eight hours. Thyroids were hydrolyzed for ten to twenty-four hours at 38° C. in 0.5 cc. of Ringer's bicarbonate buffer (pH 8.8) by 10 mg. of pancreatin, with propylthiouracil and toluene added. Six lambda of these solutions were chromatographed in butanol-acetic acid-water (75-10-15) and collidine-NH^3 for fifteen hours. The strips were scanned by a thin end-window tube and exposed to x-ray films. Levels of mono- and diiodotyrosine at twenty-four and forty-eight hours were lower than the controls at three to six hours in rats treated with thiocyanate, propylthiouracil, resorcinol, thiosulfate, chloramphenicol, and sulfaguanadine, indicating at the dose levels used, a failure to block the conversion of mono- and diiodotyrosine to thyroxine. When TSH was injected in propylthiouracil-treated rats, the conversion of mono- and diiodotyrosine to thyroxine was accelerated. The addition of iodide to the sulfaguanidine-treated rats did not inhibit, and to the propylthiouracil-treated rats seemed to accelerate the conversion of mono- and diiodotyrosine to thyroxine. This evidence indicates that the oxidative coupling of diiodotyrosyl radicals to form thyroxine differs greatly in sensitivity to antithyroid substances from the oxidative conversion of iodide to iodotyrosines.

76. Studies of the Thyroid Hormone: Triiodothyronine.

*Paul Starr, M.D.** and *B. Kallman.* School of Medicine, University of Southern California, Los Angeles, Calif.

Work was performed in this laboratory to determine the interrelationships of thyroxine and triiodothyronine. One phase of this work consisted of administering I^{131}-labeled thyroxine to athyreotic individuals and subjecting them to mild forms of stress. Using athyreotic human beings, maintained at a euthyroid level with L-thyroxine, chromatographic techniques showed no labeled triiodothyronine in butanol extracts of serum following intravenous injection of labeled thyroxine. Production of a mild stress with epinephrine did not alter this picture. Studies were also made on serum obtained from patients treated with destructive doses of radioiodine. In most cases only labeled thyroxine was found in the chromatograms of butanol extracts. In several cases, however, including 2 of hyperthyroidism and 1 of thyroid cancer, small but significant amounts of radioactivity were found in positions corresponding to triiodothyronine. In 1 case of hyperthyroidism with a gland markedly resistant to therapy, very large triiodo-

thyronine peaks were present on the chromatograms. At seventy-two hours after I^{131} treatment there appeared to be equal amounts of thyroxine-I^{131} and triiodothyronine-I^{131}.

77. Studies on the Thyroid Hormone: Sodium Dextro-Thyroxine.

*Paul Starr, M.D.** and *Ruth Leibhold-Schueck.* School of Medicine, University of Southern California, Los Angeles, Calif.

The levo and dextro-isomers of thyroxine have been made available by Dr. Leonard Ginger of Baxter Laboratories, Morton Grove, Illinois. The specific rotations of the thyroxine isomers were equal in magnitude (approximately 6.5°) but opposite in sign, suggesting that each was reasonably free of the other. Their metabolic effects were compared in myxedematous patients and in cretins. Dextro-thyroxine medication was accompanied by: 1) a rise of basal metabolism, 2) growth, with ossification of bone, and 3) electrocardiographic changes. About five times as much dextro- as levo-thyroxine was required to produce these results. The most important and unexplained phenomenon observed was that dextro-thyroxine as daily oral medication in myxedema was associated with about one third the serum protein-bound iodine concentration observed following the same amount of levo-thyroxine. Three or more times as much oral dextro-thyroxine was needed to produce the same serum protein-bound iodine level as that observed following sodium levo-thyroxine. The basal metabolic rate associated with any level of circulating thyroxine (serum PBI) was the same, whether the medication given to produce this level was levo- or dextro-thyroxine. It is possible that the serum thyroxine produced by administration of the dextro-thyroxine is actually levo-thyroxine; conversion of the dextro-thyroxine to the levo form may have occurred *in vivo.*

78. Studies on Thyroid Function in B.C.G.-Vaccinated Guinea Pig.

*J. Sternberg, M.D.** and *A. Frappier, M.D.* Institute of Microbiology and Hygiene, University of Montreal, Montreal, Canada

Studies of experimental tuberculosis and of thyroid function in guinea pigs have shown an increase of resistance to infection in hyperthyroidized animals. The thyroidal activity during the immunization process with B.C.G. has thus far not been investigated. The tissue distribution of I^{131} was studied in three groups of guinea pigs (I (80 animals); II and III (each, 60 animals)) with initial weights of 300 Gm. The first group was vaccinated with B.C.G. (60 mg. wet weight, dorsal scarification), the second with T.A.B. (intramuscular and subcutaneous route); and a third control group was scarified with an inert irritant (Amphojel). At 15-day intervals during three months after vaccination, homogeneous groups of 18 animals were injected with I^{131} and killed forty-eight hours later. The thyroidal uptake and the tissue distribution of I^{131} were determined parallel to the agglutination titer. In animals examined fifteen days after vaccination, the thyroidal uptake of I^{131} was not significantly changed in the B.C.G. group, but the tissue distribution of the isotope suggested an increased utilization of hormonal iodine (B.C.G. /controls ratio: serum PBI = 140 per cent, muscle = 290 per cent, liver = 275 per cent, bile = 315 per cent, adrenals = 340 per cent). In the T.A.B. group, the tissue concentration of I^{131} did not vary significantly from that of the controls, even during the peak of immunization when the agglutination titer was as high as 1/1520.

79. The Value of Thyrotropic Hormone in the Differentiation Between Primary and Secondary Myxedema.

Joan M. Vale, M.D., M. A. Ogryzlo, M.D. and *M. W. Johnston, M.D.* (introduced by R. F. Farquharson, M.D.). Department of Medicine, University of Toronto, Toronto, Canada

The differentiation between primary thyroid myxedema and myxedema secondary to anterior-pituitary disease can usually be made on clinical evidence. Occasionally, this differentiation may be difficult because of severe thyroid atrophy occurring in some cases of pituitary insufficiency, adrenocortical suppression in primary myxedema, thyroid suppression in Addison's disease, or in isolated instances of thyrotropin deficiency. The value of thyrotropic hormone as an aid to diagnosis has been studied in a group of 35 subjects, including 20 patients with pituitary insufficiency, 4 patients with primary myxedema, 1 patient with exogenous thyroid suppression, and 10 normal subjects. A comparison was made between single and multiple intramuscular injections of TSH, as measured by the thyroidal uptake of I^{131} and the level of serum protein-bound iodine. Changes in serum cholesterol concentration, urinary FSH values and adrenocortical stimulation with ACTH were studied in a number of instances. No demonstrable change was observed in the patients with primary myxedema, but all the normal subjects responded well. In pituitary insufficiency the response was variable. Many patients failed to show a significant response to a single injection of 12.5 mg. of TSH and 2 patients failed to respond to multiple (five) daily injections.

80. Phosphate Clearance in Parathyroid Disorders.

E. L. Chambers, Jr., M.D. and *Gilbert S. Gordan, M.D.** Endocrine Clinic, Department of Medicine, University of California School of Medicine, San Francisco, Calif.

Simultaneous determinations of glomerular filtration rate by endogenous creatinine clearance and of renal excretion of phosphate permit calculation of reabsorption of phosphate (Schaaf and Kyle: *Am. J. M. Sc.* **40:** 765, 1954). The phosphate/creatinine clearance ratio (Fraser and Nordeen: personal communication) yields approximately the same information. We have measured phosphate reabsorption in 4 patients with hyperparathyroidism, 4 patients recovering from hyperparathyroidism, and 5 patients with treated hypoparathyroidism. Normally, 82–93 per cent of filtered phosphate is reabsorbed. Hyperparathyroid patients resorbed less than 81 per cent. Hypoparathyroid patients receiving vitamin D or dihydrotachysterol showed normal rates (81–95 per cent). In 2 patients after parathyroid adenomectomy, the rate rose (up to 99.7 per cent) and slowly returned to normal. One patient with advanced skeletal involvement showed a reduced rate postoperatively, probably because of soaking up of phosphate by the "hungry bones." Another patient without evident osseous involvement (normal alkaline phosphatase values and normal skeletal roentgenograms) showed a similar postoperative defect. These data support Albright's thesis that excess parathyroid hormone induces phosphate diuresis.

81. Hypophosphatemic Rickets Associated with Slight Acidosis.

Gunther A. Fromm, M.D., Jaime Roca, M.D. and *Eduardo Fideleff, Bioch. D.* (introduced by Eugenia Rosemberg, M.D.). Endocrinological Department of the Instituto de Cirugia of the Province of Buenos Aires, Argentina.

The case of an 8-year-old girl (height 1.05 M., weight 24.7 Kg.) hospitalized for genu valgum is discussed. The roentgenograms at first suggested chondrodystrophy, but rickets was noted in the wrists. Serum phosphorus concentration was 2.28 mg. per 100

ml., alkaline phosphatase 16 Bodansky units, serum calcium 11.2 mg. per 100 ml., and blood bicarbonate 23.8 mEq/L. Urine pH was 6 and the average daily excretion of calcium was 35 mg.; phosphorus 455.5 mg.; ammonia 0.43 Gm. N; bicarbonate 5.68 mEq.; "titrable acidity" 0.57 mEq./NaOH/Kg.; and aminoacid nitrogen 121.9 mEq. Insulin clearance was 102.1 ml./minute. Average daily phosphorus excretion in feces was 81.8 mg.; fat content 18.5 Gm. per cent. Results of an Ellsworth-Howard test were normal. Although tubular phosphorus reabsorption amounted to 92 per cent, the phosphorus clearance was considered high in relation to the serum phosphorus level. Hypophosphatemic rickets associated with hypocalciuria and slight acidosis was diagnosed. Steatorrhea and hyperparathyroidism could be excluded as etiologic factors. Treatment began with 50,000 calciferol units daily, progressively increased to 350,000, together with 50 ml. of Shohl's citric-acid sodium-citrate mixture, to: a) correct the acidosis, and b) facilitate deposition of calcium in bone and prevent hypercalcemia. After eighty-two days of treatment, roentgenograms showed normalization, and a serum phosphorus level of 5.8 mg. per 100 ml. Calciferol was lowered to 180,000 units daily. It is suggested that in spite of normal excretion of ammonia and titrable acidity, the case was one of rickets due to renal tubular insufficiency.

82. Pseudofractures with Hypercalcemia: Osteomalacia *vs.* Hyperparathyroidism.

Irvin C. Plough, Maj. MC, USA and *Laurence H. Kyle, M.D.** Department of Metabolism, Walter Reed Army Medical Center, and Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

A 23-year-old man presented the unique combination of osteomalacia and hypercalcemia. Initial study showed decreased bone density, pseudofractures, hypophosphatemia, elevated phosphatase, and hypercalciuria, but the serum calcium level was persistently elevated. Bone biopsy showed osteomalacia; exploration of the neck disclosed two hypoplastic parathyroid glands. Except for increased phosphate clearance there was no evidence of defective renal tubular reabsorption nor of impaired urinary acidification. There was no increase in the low rate of phosphorus reabsorption during phosphate infusion. Intravenous administration of calcium was associated with a moderate rise in the level of serum phosphorus but there was no decrease in total phosphate excretion on the day of the infusion; calcium retention was subnormal. Balance study demonstrated almost complete gastrointestinal absorption of calcium. Added phosphorus produced positive calcium and phosphorus balance, with a marked decrease in urinary calcium. Increased calcium intake also caused calcium and phosphorus retention but the highest degree of positive balance was attained with a diet high in both calcium and phosphorus. Large doses of vitamin D caused no change in calcium or phosphorus excretion. Although either hyperparathyroidism or a renal tubular reabsorptive defect might result in certain of these abnormalities, neither diagnosis offers an adequate explanation for the entire picture.

HYPOTHALAMUS AND PITUITARY

83. Some Observations on Water and Electrolyte Excretion in Rats with Diabetes Insipidus.

Carl S. Alexander, Lieut. (MC) USNR, Great Lakes, Ill. (introduced by Edmund B. Flink, M.D.). University of Minnesota, Minneapolis, Minn.

In 15 per cent of rats subjected to neurohypophysectomy permanent diabetes insipidus (DI) developed. For more than twelve months these animals excreted 75 to 142

ml. of urine per day as compared to 8 to 21 ml. for controls. When the drinking water was replaced by 1 per cent saline, DI rats excreted 76 to 305 ml. of urine per day, whereas controls excreted 28 to 71 ml. The major action of desoxycorticosterone acetate (DCA) in both DI and controls was to reduce sodium excretion. However, the effect on potassium and chloride was sometimes in opposite directions. The antagonistic action of Pitressin and DCA on salt excretion was demonstrated. The rate of excretion of an acute water or salt load depended on the nature of the loading solution, its route of administration (by gavage or intraperitoneal), the state of hydration prior to loading, and the use of Pitressin or DCA. Water deprivation of DI rats prior to water loading by either route resulted in marked inhibition of diuresis. However, DI rats excreted a salt load more rapidly than controls under all conditions studied.

84. Localization of Glycoprotein Hormones of the Adenohypophysis by Combined Histochemical, Physiochemical and Bioassay Procedures.

*Aaron J. Ladman, PhD.** and *Russell J. Barrnett, M.D.** Department of Anatomy, Harvard Medical School, Boston, Mass.

To prove that the PAS+ granules in the "basophiles" of the adenohypophysis contain the glycoprotein hormones (FSH, LH and TSH), an attempt was made to find a solvent which would dissolve one or more of these hormones and precipitate the remainder. After such a procedure, some of the glands were studied histologically to note the presence or absence of PAS+ material in "basophiles," as well as the staining characteristics and structure of the "acidophiles." Other glands were assayed in hypophysectomized immature rats to establish the relative content of the hormones retained. No solvent tested was capable of removing one hormone alone. However, one solvent (2.5 per cent trichloracetic acid) was found, which removed FSH and TSH, but not appreciable amounts of LH, as determined by bioassay. Histologically, these glands appeared devoid of PAS+ material, except in some "central basophiles." Another solvent (50 per cent pyridine) was found to remove all three glycoprotein hormones as proven by bioassays. Under these conditions, there was no PAS+ material demonstrable histochemically in the cells of the glands. Similar studies were made on pituitaries of rats in various abnormal endocrine conditions.

85. Cortisone Inhibition of ADH Secretion From the Neurohypophysis.

Joseph F. Dingman, M.D. and *George W. Thorn, M.D.** Department of Medicine, Harvard Medical School and Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

An earlier communication (*Fed. Proc.* **13**: 36, 1954) reported a group of patients with clinical diabetes insipidus in whom studies of neurohypophyseal function revealed a normal antidiuresis following small intravenous doses of nicotine and Pitressin. However, no antidiuresis occurred with hypertonic saline. This suggested that a selective failure of osmoreceptor function existed. One of these patients also had anterior hypopituitarism with hypogonadism and hypoadrenalism but normal thyroid function. Further studies on this patient when hypothyroidism ensued revealed an increased sensitivity to the antidiuretic effect of nicotine. This was improved but not made normal by adequate thyroid and estrogen therapy. The depressed free water clearance (C H_2O) was not improved. Additional administration of 25 mg. of cortisone daily increased daily urine volume and diuretic response to water administration and abolished antidiuretic response to nicotine. Renal sensitivity to Pitressin was unaltered. Cortisone also aggra-

vated the polyuria in another patient with untreated diabetes insipidus receiving a constant electrolyte, but unrestricted fluid intake. In addition, cortisone diminished the antidiuretic response to smoking in normal subjects. These findings are consistent with the hypothesis that the diuretic effect of cortisone may, in part, be mediated by central inhibition of ADH secretion from the neurohypophysis.

86. Metabolic Actions of Pituitary Ketogenic Factor in the Rat.

*Frank L. Engel, M.D.** and *Mildred G. Engel M.S.* Duke University, Durham, N. C.

Oxycel corticotropin has been found by Astwood's group and ourselves to be a rich source of ketogenic factor when tested in intact and adrenalectomized rats. In the present study, acute effects of corticotropin on blood glucose and ketone levels were investigated during and after a 30-minute intravenous infusion of a fatty acid, sodium octanoate. A 0.12-mg. dose of corticotropin was injected thirty minutes before infusion. The changes in blood ketones during thirty minutes' infusion were as follows: (mean $\pm$S.E.) saline control, $+1.00 \pm 0.26$ mg./100 ml.; octanoate, $+2.35 \pm 0.42$ mg.; corticotropin, $+3.06 \pm 0.35$ mg.; and corticotropin+octanoate, $+6.94 \pm 0.57$ mg. Correcting for the control rise, the increase in ketones after combined treatment (5.94 mg./100 ml.) was significantly greater than the sum of either alone (3.41 mg./100 ml.), suggesting a stimulation of ketone-body formation from the fatty acid. In a second experiment, 10 per cent glucose was substituted for saline and added to the octanoate solution. The changes in ketones were as follows: glucose control, -5.49 ± 0.75 mg./100 ml.; octanoate-glucose, -4.18 ± 0.57 mg.; corticotropin-glucose, -0.90 ± 0.80 mg.; and corticotropin-octanoate-glucose, -0.20 ± 0.90 mg. Corticotropin thus abolished the antiketogenic effect of glucose. The blood glucose levels in the glucose control group before, at the end of, and thirty minutes after glucose infusion were 90 ± 2.9, 216 ± 5.1, and 119 ± 5.8 mg./100 ml., whereas in the corticotropin-glucose group they were 91 ± 2.6, 175 ± 10.0 and 84 ± 5.8 mg./100 ml., indicating accelerated glucose utilization after hormone treatment.

87. Renal and Metabolic Studies, with Autopsy Findings in a Patient with Gigantism and Acromegaly.

*Herbert Gershberg, M.D.,** *Henry O. Heinemann, M.D.* and *Harry H. Stumpf, M.D.* Departments of Medicine, Physiology and Pathology, New York University College of Medicine, and Third (N.Y.U.) Medical Division, Bellevue Hospital, New York, N. Y.

Growth hormone exerts a potent effect on carbohydrate metabolism and on kidney function in animals. The endogenous hormone has a similar effect in human beings, as indicated by the occurrence of insulin resistance and kidney hypertrophy in acromegalics. We have had the rare opportunity of studying an active acromegalic who shortly thereafter came to autopsy. A 36-year-old male, 6 feet 6 inches in height, who had had a pituitary tumor for twenty years, entered the hospital in diabetic acidosis. He required 400 units of insulin daily. The renal functions were markedly increased (normal values in parentheses); glomerular filtration rate was 325(131) cc./min.; urea clearance 159(70) cc./min.; sulfate Tm 289(70) micromols/min.; glucose Tm 1068(375) mg./min.; and TmPAH 165(76) mg./min. The values corrected for surface area were 50–100 per cent higher than normal. At autopsy four weeks later, the heart weighed 900 Gm., the right kidney 600 Gm., and the left kidney 550 Gm. The pancreas did not exhibit any

pathologic lesions. On preliminary examination of the kidney, Dr. Jean Oliver estimated the diameter of the glomerulus as 333 micra (normal 212), and that of the proximal convoluted tubule as 85 micra (normal 65).

88. Studies of the Antidiuretic Content of the Hypothalamus of Intact and Hypophysectomized Rats.

*Charles W. Lloyd, M.D.** and *Patricia Oldford, B.A.* State University of New York Upstate Medical Center, Syracuse, N. Y.

Antidiuretic activity of rat hypothalami, including the median eminence, was measured by intravenous administration of homogenates to alcohol-anesthetized, hydrated rats. Hypothalami of intact, hydrated (*ad lib.*), adult male Sprague-Dawley rats contain from 10 to 18 milliunit equivalents of vasopressin. Moderate dehydration (forty-eight hours) results in an increase, whereas intracarotid administration of hypertonic saline solution causes a decrease of antidiuretic activity of the hypothalamus. Maintenance for two or three days on a liquid diet results in a decrease in hypothalamic antidiuretic content. These studies suggest that the hypothalamic content of antidiuretic material reflects changes in the rate of release and formation. Hypothalami of animals hypophysectomized for thirty days contain 10 milliunit equivalents of vasopressin, whereas pretreatment with hydrocortisone increases the content by at least ten times. These findings are compatible with the hypothesis that the neuro-secretory material of the hypothalamic tract is either identical with, or closely associated with the neurohumoral substance causing ACTH secretion by the anterior lobe.

89. Hormonal Influences Upon Mast Cells.

Jacques Padawer, Ph.D. and *Albert S. Gordon, Ph.D.** Department of Biology, Graduate School of Arts and Science, New York University, New York, N. Y.

It has been shown that the mast cells of rat peritoneal fluid undergo degenerative morphologic changes following hypophysectomy, and are not affected by adrenocortical factors (Padawer and Gordon; 1953, 1954). Growth hormone (Raben-Westermeyer GH) overcomes these mast-cell changes in the hypophysectomized rat. In the present study, GH(500 μg./day) and cortisol (1 mg./day), alone and in combination, were administered for two weeks to hypophysectomized rats. As may be noted from the Table, GH administered alone inhibited the mast-cell changes following hypophysectomy, an influence that was prevented by simultaneous administration of cortisol. Vehicle-injected controls showed no alterations in either mast-cell size or numbers of abnormal mast-cell forms, the 2 parameters found to be related to pituitary function.

	Hypophysectomized				
Mast cells	*GH*	*Cortisol*	*GH+ Cortisol*	*Controls*	*Not operated on*
Size (units)	5.56*	6.38	6.74	6.45	5.40
Abnormal shapes (%)	5.94*	17.1	16.7	14.4	1.70

* Statistically significant *cf.* hypophysectomized controls.

90. The Effect of Sodium Chloride Intake on Fluid Retention in Rats on Low-Sodium Diets.

*Elaine P. Ralli, M.D.** and *Bertram Laken, M.S.* Department of Medicine,

College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Seventy-day-old male rats were given diets containing 12 per cent protein, 73 per cent carbohydrate and 10 per cent fat. The animals were divided into two groups, one group being allowed 1 per cent NaCl solution *ad lib.* and the other group receiving distilled water. No sodium chloride was added to the diet, so that the rats on distilled water were on a relatively salt-free diet. At intervals of about ten days, water tolerance tests were carried out. The rate of urine excretion and the excretion of sodium, chloride and potassium were measured. After fifty-five days on the diet, 24-hour urine specimens were collected in both groups of rats. The urines were assayed for their antidiuretic effect. After seven days on the diet, water tolerance was impaired in the rats on the low-sodium intake. The values became progressively more depressed as the experiment continued. The capacity to handle a water load was not impaired in the rats on the high-salt intake until towards the end of the experiment. The urine of the rats on the low-sodium intake had a pronounced antidiuretic effect when injected into hydrated rats. The urine from the rats receiving sodium chloride had only a moderate antidiuretic effect, on assay.

REPRODUCTION AND SEX HORMONES

91. Difference in Response of the Normal and Intrasplenically-Grafted Ovary to Gonadotropin and Estrogen.

*E. C. Bernstorf, Ph.D.** Department of Anatomy, Hahnemann Medical College, Philadelphia, Pa.

The intrasplenically-grafted ovary becomes tumorous, even though a significant amount of its secretion apparently escapes through the liver into the general circulation. The present experiment was set up to help elucidate the endocrine imbalance in the graft-bearing animal and to compare the response of the graft with that of the normal ovary. Mice were operated on at 55 to 65 days of age and a fourteen-day period of treatment with various dosages and combinations of FSH (Gonadogen), LH (Antuitrin-S) and estradiol was started thirty days later. Each gonadotropin treatment increased the ovarian and utero-vaginal weights in animals not operated on, but decreased ovarian-graft weights in the graft-bearing groups (significant for $FSH_{17}LH_{15}$). (Subscripts refer to the daily dose in international units.) Of the nonestrogen-treated graft-bearing animals only the FSH_8 and $FSH_{17}LH_{15}$ groups showed stimulation of graft secretory activity (indicated by increased utero-vaginal weight). Gonadotropin stimulated formation of large follicles (hemorrhagic and "cystic") in ovaries but decreased these structures in ovarian-grafts.

92. The Effect of Reserpine Alone and in Combination with Estrogen and/or Testosterone in the Climateric.

Meyer H. C. Blatt, M.D., Hans Wiesbader, M.D. and *Herbert S. Kupperman, Ph.D., M.D.** Department of Therapeutics, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

A series of well established and controlled menopausal patients, both natural and surgical, were treated with different combinations of reserpine with or without estrogen and/or testosterone. The response to standard estrogens was well documented. The menopausal-index method of evaluation, utilizing the double blind testing technique, was employed. A dose of 0.1 to 0.2 mg. of reserpine was administered twice per day,

combined with the hormone preparations—ethinyl estradiol, methyltestosterone, and diethylstilbestrol. The data did not indicate that reserpine effectively enhanced the value of the endocrine preparations in the treatment of the climateric. On the other hand, a fixed combination of reserpine with estrogen and/or androgen, as indicated, resulted in an inflexible preparation not ideally suitable for therapy. Alteration of dosage of the individual components was not possible. Undesirable side effects due to reserpine were noted in a few patients and were characterized by untoward dreams, nasal stuffiness and excessive sedation. Libido was not adversely effected or benefited by the reserpine. It was concluded that although reserpine might be of some value in the menopause due to its sedative qualities, fixed combinations with various estrogenic and/or androgenic preparations did not appear to be the most propitious form of therapy.

93. Effect of Progesterone on the Induction of Leydig-Cell Tumors With Estrogen in Strain A Mice.

Ben B. Blivaiss, Ph.D., R. O. Hanson, Ph.D., R. E. Rosenzweig, B.S.* and *H. Kutuzov, M.S.* Department of Physiology and Pharmacology, The Chicago Medical School, Chicago, Ill.

Gardner (*Anat. Rec.* **68**: 339, 1937) postulated that estrogens elicit an increased production of pituitary interstitial-cell stimulating hormone (ICSH), which determines the development of testicular interstitial-cell tumors. Since progesterone is known to have an inhibitory effect on production and/or release of LH or ICSH, a comparison was made of the effects of separate and combined injections of diethylstilbestrol (DES) and progesterone (P) on development of interstitial-cell tumors. A group of 175 strain A mice, 4–5 weeks old, was divided into the following treatment categories: 1) 0.05—0.25 mg. DES per week, 2) 1.25 mg. P weekly, 3) combination of DES and P as described, and 4) 0.05 cc. sesame oil weekly. Of 55 DES-treated mice examined at autopsy after 4 months of age, when the first tumor appeared, 76 per cent had tumors, of which 5 were unilateral and 37 were bilateral. After 9 months of age, all mice had bilateral tumors. Of 45 DES- and P-treated mice examined at autopsy after 4 months of age, 45 per cent had tumors, of which 9 were unilateral and 14 were bilateral. The first tumor was not found until 7 months of age. Mice receiving progesterone showed only an underdevelopment of interstitial cells, both as to number and size, up to 6 months of age; but thereafter they showed normal development.

94. Histologic Study of Ectopic Testes During Puberty.

F. A. de la Balze, M.D., O. Vilar, M.D., F. Arrillaga, M.D., A. I. Gurtman, M.D.* and *R. E. Mancini, M.D.* IIIa. Catedra de Clinica Medica de la Facultad de Medicina, Buenos Aires, Argentina.

From the histologic point of view, 7 cases of ectopic testes have been studied during puberal development. Comparatively, the eutopic testes of each patient were examined. Histologic and histochemical techniques were applied.

RESULTS: The eutopic testes presented changing degrees of maturation which, according to a previous study of ours, were classified as: almost immature, semi-mature, and almost mature. In the ectopic testes there appeared alterations in the tubular content, tubular wall and in the intertubular connective tissue. These alterations were of two types: a) delay in the maturation; b) pathologic maturation. When the eutopic testis was almost immature, the ectopic one presented mainly a delay in maturation and only slight manifestations of pathologic maturation. When the eutopic testis was

almost mature (similar to that of an adult), the ectopic one showed a delay in maturation and distinct manifestations of pathologic maturation. The latter consisted of: a) spermatogenic abnormalities; b) sclerosis and fibroblast proliferation in the tubular wall, and c) abundant gigantic Leydig cells, with intense cytoplasmatic vacuolization and nuclei with variable degrees of pyknosis. The results of histochemical studies are presented. Presumably, the delay in maturation is chiefly the result of the ectopic position, whereas the pathologic maturation is the result of the puberal stimulus of growth acting on the ectopic testes.

95. The Vaginal Smear As A Method of Endocrine Evaluation: Method and Preliminary Results.

John A. Finkbeiner, M.D. (introduced by Rulon W. Rawson, M.D.) Memorial Center and Sloan-Kettering Institute for Cancer Research, New York, N. Y

In 1933 Papanicolaou introduced the vaginal smear as a method of endocrine evaluation in the human female. In the clinical application of this technique it has been customary to describe the smear by the degree of cornification present, *i.e.*, in terms expressing maximal activity. Instead, it is proposed to consider the basal cell as the fundamental cell of the vaginal smear; therefore epithelial cells other than the basal cell must represent some degree of stimulation.

A series of patients has been studied—normal females, women with various endocrinopathies, and a large group of patients with breast cancer before, during, and after endocrine therapy, both ablative and additive. Periodic vaginal smears have been prepared by a modified Papanicolaou technique and a differential count performed of the epithelial cells, recording the percentage of cornified, noncornified, pyknotic, intermediate, para-basal, and basal cells in each smear. Preliminary results indicate that this method is sufficiently sensitive to detect the single injection of 0.5 mg. of estradiol. The cases demonstrate a range of variability in the vaginal-smear pattern that may be seen in normal regularly-menstruating females, in the premenopausal and postmenopausal states, and as a result of various diseases and/or therapy.

96. Renal β-Glucuronidase Response to Gonadotropins.

William H. Fishman, Ph.D., George Benjamin, A.B.* and *Sidney Green, B.S.* Tufts College Medical School, Boston, Mass.

Previously, it was noted that a number of androgens were capable of producing a marked increase in renal β-glucuronidase activity when administered under standard conditions to mice of both sexes of the Ajax strain. This is a report of a study of the influence of anterior pituitary hormones on kidney β-glucuronidase. The following preparations had no effect on kidney β-glucuronidase in male mice: growth hormone, ACTH, thyroid, thyroxine and prolactin. Thyrotropin and gonadotropin did stimulate the renal enzyme. It was possible by experiment to explain the action of thyrotropin on the basis of its contamination with gonadotropin. Every preparation of gonadotropin, whether of pituitary, serum or urinary origin, showed positive results only in male mice, apparently by stimulating androgen production of the interstitial cells of the testis. The activity of sheep pituitary gonadotropin appears to be a property solely of luteinizing hormone. Linear curves were obtained relating renal β-glucuronidase response with log dose of gonadotropin, or of LH. This may provide the basis of a more specific bioassay of LH and may also explain sex differences in kidney β-glucuronidase of certain mouse strains.

97. Effects of X-Irradiation on the Response of Accessory Sex Organs to Androgens.

*Walter Fleishmann, M.D.** and *Carl E. Nurnberger, Ph.D.* V. A. Hospital (Crump) and V.A.M.T.G. Hospital, Memphis, Tenn.

X-irradiation of the accessory sex organs of the castrated male rat and subsequent treatment with testosterone propionate results in cellular hypertrophy with delayed onset of mitosis under certain experimental conditions (Fleischmann and Nimaroff: *Proc. Soc. Exper. Biol. & Med.* **85**: 655, 1954). These experiments were continued, using castrated male mice and testosterone cyclopentylpropionate (TCP). Mice received doses ranging from 3000 to 5000 r to the pelvic region and were given 0.3 mg. of TCP parenterally immediately afterwards. Controls received TCP only. The seminal vesicles were removed forty-eight hours later. Mitotic activity was suppressed in the irradiated specimens in contrast to the controls. This contrast could be demonstrated well by the colchicine technique. Although mitotic activity was suppressed, the irradiated cells were transformed from the cuboidal type to the secretory columnar type. Their cytoplasma was highly basophilic. This basophilic reaction was no longer obtained after selective destruction of the ribose nucleic acid (RNA) by the method of Fisher (*Stain Technol.* **28**: 9, 1953). The findings suggest that under the conditions of these experiments, x-irradiation does not inhibit synthesis of RNA, but rather the formation of molecular components necessary for mitosis. This inhibition was found to be reversible.

98. The Eunuchoid Female: A Case Study.

*Robert B. Greenblatt, M.D.** and *Nicanor Carmona, M.D.* Medical College of Georgia, Augusta, Ga.

This is a case study of a white female 25 years of age, tall and eunuchoid in appearance, with primary amenorrhea, failure of breast development, an infantile uterus, scanty pubic hair growth, and a markedly enlarged clitoris. There were no signs of hypertrichosis. The chromosomal sex skin was of the male type. The vaginal smear was atrophic. The urinary 17-ketosteroids were decreased; the urinary FSH value was within normal limits. The bone age was delayed. A menstrual period was induced by estrogen-progesterone medication. Laparotomy revealed the presence of rudimenatry gonads. On histopathologic study, the cortex was composed of ovarian stroma without primordial follicles, and the medulla and hilus contained many focal areas of Leydig cells. This is another unusual case of ovarian dysgenesis with androgenic phenomena, and is worthy of report.

99. Allergenic Properties of Pregnanediol.

*George P. Heckel, M.D.** and *Henry W. Scherp, Ph.D.* University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Investigation of the allergenic properties of pregnanediol was prompted by clinical evidence of endogenous allergy to steroids, notably pregnanediol. (*Surg. Gynec. & Obst.* **29**: 191, 1951; *Am. J. Obst. & Gynec.* **66**: 6, 1297, 1953). Rabbits and guinea pigs were given sensitizing injections of pregnanediol in mineral oil, and pregnanediol in mineral oil plus killed tubercle bacilli with and without a wetting agent, in single and in divided doses. Skin sensitivity was produced in 20 to 80 per cent of animals by the various techniques. Sensitivity was tested by intracutaneous injections of microcrystalline suspensions of pregnanediol in normal saline. Positive skin reactions were elicited with as little as 0.1 milligram. The higher percentages of sensitized animals were obtained when killed tubercle bacilli were used as an adjuvant. There was no correlation of

sensitivity to pregnanediol with the tuberculin sensitivity in animals which received killed tubercle bacilli. Some animals showed initial sensitivity of the skin to pregnanediol, which was increased by sensitization. Male animals appeared to be more easily sensitized to pregnanediol than female animals.

100. The Behavior of Experimental Hydro-Uteri in Rodents.

F. Homburger, M.D., Milton S. Grossman* and *A. Tregier.* Cancer Research and Cancer Control Unit, Tufts College Medical School, Boston, Mass.; and Jackson Memorial Laboratory, Bar Harbor, Me.

Ligation of the cervix uteri in rodents caused marked distention of uterine horns, resulting in huge uteri resembling those found in mice with imperforate vagina. The development of this hydro-uterus depended upon several factors, such as strain of the animals, age, and endocrine status. Studies were made of the effects of castration and hormone administration upon the rate of development and histologic appearance of hydro-uteri. The experimental hydro-uterus provided a means for the study of uterine secretions of rodents, since the chemical composition indicated that this secretion was neither an exudate nor a transudate, but more likely a collection of endometrial secretions. The hydro-uterus was also an excellent site for the transplantation of tumors in rodents.

101. Adrenal Facilitation of the Early Growth of the Genital Tract.

Evelyn Howard, Ph.D. (introduced by George W. Corner, M.D.). Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

Attempts of the author and others to detect a physiologically appreciable degree of androgen production by the adrenals of mice have hitherto been unsuccessful, in that no short-term effect of adrenalectomy on the seminal vesicles of castrated mice could be observed. Comparable experiments in rats revealed only a brief prepuberal period when there is a detectable effect of adrenalectomy on the ventral prostate. However, if mice are adrenalectomized at 12 days of age, the gonads being left intact, and maintained on small amounts of desoxycorticosterone, body growth is nearly normal during a period of twofold increase, but relative growth of the seminal vesicles or uterus is reduced by half. These defects in substitution therapy on desoxycorticosterone were prevented in either sex by the simultaneous administration of dehydroepiandrosterone, but other organ weights were not normal. Dehydroepiandrosterone administered to gonadectomized animals produced much less growth of vesicles and uteri. The biologic properties of dehydroepiandrosterone appear to satisfy certain requirements of a component of the adrenal hormone complex in this experimental situation.

102. Surgery as an Adjunct to Hormone Therapy of Inoperable Breast Cancer.

*B. J. Kennedy, M.D.** and *Ira T. Nathanson, M.D.* University of Minnesota Medical School, Minneapolis, Minn., and Massachusetts General Hospital, Boston, Mass.

There has been an increasing awareness during recent years that certain cancers of the breast are not curable by radical operative procedures. The criteria of operability have become more strict and a greater number of patients are being deemed inoperable. Hormonal therapy is of value in the treatment of inoperable carcinoma of the breast. In many instances the regressions in selected patients have equalled or excelled those seen after radiation therapy. Observations indicate that a certain number of advanced

primary cancers of the breast originally deemed inoperable, became localized during hormonal therapy. Twenty such patients were subjected to surgical resections of the breast, to obtain pathologic material for study of the mechanisms of regressions. The subsequent clinical course was unusually favorable. Three patients were alive without evidence of recurrent disease more than seven years after the onset of hormone therapy. Four patients died of cardiovascular diseases without apparent recurrent cancer ten months, and two, four and eight years after initiating therapy. The remaining patients died of metastatic breast cancer between two and six years from the onset of hormone therapy. It appears that surgical procedures are of value as an adjunct to hormonal therapy of advanced cancer of the breast. As far as palliation is concerned in this group of patients, the combination of techniques may offer more than either surgery or hormonal therapy alone.

103. The Protein Catabolic Effect of Progesterone.

*Laurence H. Kyle, M.D.** and *Walter C. Hess, Ph.D.* Departments of Medicine and Biological Chemistry, Georgetown University School of Medicine, Washington, D. C.

Although occasional mention has been made of an apparent effect of progesterone on nitrogen balance, only recently has this subject been afforded study. The present investigation concerns measurement of nitrogen excretion during progesterone administration under varying conditions. The administration of progesterone and anhydrohydroxyprogesterone to 4 men and 4 women with rheumatoid arthritis and apparently normal adrenal function resulted in slight but consistent increase in nitrogen excretion. There was no change in eosinophils, lymphocytes, or blood glucose and serum cholesterol levels to suggest a cortisone-like action. When given immediately following two weeks of ACTH therapy, progesterone induced the usual degree of increased nitrogen excretion. A similar effect of progesterone, although somewhat more marked, occurred when it was given following the use of cortisone. Urinary 17-ketosteroid excretion was not significantly affected by progesterone or anhydrohydroxy-progesterone in any of the 10 patients studied. In a case of congenital adrenal hyperplasia there was no depression of the elevated 17-ketosteroid excretion, despite the fact that nitrogen excretion increased significantly. These studies indicate a moderate but consistent protein catabolic effect of progesterone, possibly attributable to suppression of "N-hormone" production but more probably the result of direct action at the tissue level.

104. Influence of Ovariectomy and Castration on Development of Experimental Fungus Injections in Mice.

Zbigniew T. Mankowski, M.D. (introduced by J. J. Rupp, M.D.). Institute for Cancer Research, Philadelphia, Pa.

Our previous observation that estradiol enhances fungus infections in mice suggested that ovariectomy might also influence this type of infection. Because one might expect that chronic infections would show this effect more conspicuously, we used a suspension of *Nocardia mexicana* which usually kills mice regularly when injected with 1 cc. of a 1:100 mucin suspension intraperitoneally in a period of seven to fourteen weeks. The survival time of ovariectomized mice infected in this way with *Nocardia mexicana* was distinctly longer than that of controls. In more acute infections produced by intravenous injection of 0.2 cc. of a 1:500 suspension of *Cryptococcus neoformans* and 0.2 cc. of a 1:1600 suspension of *Candida albicans* the same results were observed. Ovariectomized

mice appear to have a higher leukocyte count than the controls, which would agree with our observation previously reported that estradiol-treated mice had a lower leukocyte count than the control, fungus-infected mice. After prolonged estradiol treatment, our experimental mice showed a very high percentage of distention of the urinary bladder. Such observations have been made in the past by Lacassagne and Burrows. Castration does not significantly influence the course of experimental fungus infection. Estradiol seems to have a more deleterious effect on castrated than on ovariectomized mice.

105. The Specificity of the Weaver-Finch Test for the Luteinizing Gonadotropins (LH and CGH).

S. J. Segal, Ph.D. and *Emil Witschi, Ph.D.** Urology and Zoology Departments, State University of Iowa, Iowa City, Ia.

The claim of specificity of the weaver-finch reaction in the assay of luteinizing hormones is based, above all, on its sensitivity to pregnant mare serum. Since the finch reacts negatively to serum of the nonpregnant horse, only the prevalent FSH or the associated LH principle can be the cause of positive reactions. Tests with hypophyseal preparations show that LH and not FSH is the active agent. In earlier experiments it had been found that the gonads of birds, like those of mammals, are relatively insensitive to chorionic gonadotropin. The latter material being of luteinizing type, one might expect the weaver-finch feather to respond easily to its administration. This is not actually the case, as only doses above 60 I.U. cause positive reactions. Thus the weaver feather reacts positively to about .1 to .01 R.U. of most hypophyseal preparations, to 10 R.U. of pregnant mare serum, and to 60 R.U. of human chorionic gonadotropin. Evidently these differences reflect in part the dependence of the R.U. on the FSH content of the various preparations. It may be questioned whether the finch reaction directly measures the true amount of LH fractions or whether it depends partly on molecular structure and availability of active groups.

106. The Respiration of Slices of Mammary Gland from Pregnant and Lactating Guinea Pigs.

Thomas C. Smith, Ph.D. and *B. Richterich.* Department of Pharmacology, Boston University School of Medicine, Boston, Mass.

The endogenous respiration of mammary gland slices and their response to the addition of various substrates has been studied with the Warburg technique. Slices prepared on a Stadie Riggs microtome were collected in ice-cold Krebs-Ringer-phosphate medium (pH 7.4). After 10′ equilibration in the same medium at 37.5° C. in air, substrates were tipped from the sidearm to make a final concentration of 20 mM. Readings were taken over the period of an hour or more. QO_2DW, increased significantly ($P < 0.001$) after parturition, was maintained through the tenth day, but had declined by the twentieth day post partum. Concomitantly, the response to added pyruvate, acetate, and succinate, was enhanced. Glucose, ketoglutarate, fumarate, and citrate were without significant effect. The data showed the same general trend, whether calculated on the basis of tissue fresh-weight, defatted dry-weight, total nitrogen, protein, or DNA. The metabolism of other organs was also studied for comparative purposes.

107. The Metabolism of Radioactive Estradiol in Men.

*Benjamin F. Stimmel, Ph.D.** and *James A. May, M.D.* Rees-Stealy Clinic Research Foundation, San Diego, Calif.

A single 0.2-mg. dose (600,000 c.p.m.) of estradiol-16-C^{14} was administered orally to men, and the 48-hour post-treatment urine was collected. Aliquots were treated as follows: 1. Boiled with 15 per cent v/v concentrated hydrochloric acid for twenty minutes. 2. Boiled with 15 per cent v/v concentrated hydrochloric acid and 4 Gm. per cent of zinc dust for three hours. 3. Incubated for forty-eight hours at pH-6 and 37° C. with β-glucuronidase (bacterial). Neutral and phenolic fractions of the ether-extractable material were prepared after the foregoing treatment. The phenolic fractions were processed further by our physicochemical procedure for estrone, estradiol, and estriol (*Fed. Proc.* 9: No. 1, March, 1950). In our first experiment, aliquot 1 indicated 0.8 and 20 per cent respectively of the administered activity in the 48-hour neutral and phenolic fractions. Aliquot 2 indicated 0.9 and 25 per cent respectively in similar fractions. Estrone accounted for 40, estradiol 7, and estriol 43 per cent of the activity of phenolic fraction 1. Similarly, phenolic fraction 2 indicated 6, 33 and 41 per cent respectively. In our second experiment, hydrolytic procedure 1 indicated 1.2 and 31 per cent respectively of the administered radioactivity in the 48-hour neutral and phenolic fractions. Hydrolytic procedure 3 indicated 1.6 and 33 per cent respectively. Fractionation of phenolic residue 1 indicated 76, 9 and 6 per cent, and fractionation of phenolic residue 3 produced 70, 9 and 9 per cent respectively of estrone, estradiol, and estriol.

108. Comparative Evaluation of Various Types of Administration of Progesterone.

*Edward T. Tyler, M.D.** University of California, School of Medicine, Los Angeles, Calif.

The author has evaluated the use of the following methods of progesterone administration in infertility practice: oral, transmucosal, intramuscular, vaginal and rectal. Therapeutic effectiveness was judged on the basis of several criteria, including endometrial changes, basal body temperature observations, and pregnanediol assays. These criteria often fail to correlate in evaluating therapy during a given cycle. For example, high levels of pregnanediol excretion, as obtained with oral medication, are not indicative of good physiologic effect as demonstrated by endometrial changes. Vaginal administration of progesterone was found to prove most useful when the required dosages are so high that they would ordinarily produce severe local reactions by intramuscular injection. Clinical use of a new oral progestogen, 19-norethinyl testosterone is described. This preparation was found to have marked thermogenic, and other physiologic effects in comparatively small dosage.

109. Effects of Parenteral, Oral, Buccal and Vaginal Progesterone on the Endometrium.

*M. James Whitelaw, M.D.** Department of Obstetrics and Gynecology, University of California School of Medicine, San Francisco, Calif.

Twenty-two infertility patients with secondary amenorrhea, after a preliminary seven-day estrogen priming, were given for ten consecutive days varying doses of oral estrogen (0.5–5.0 mg.) and progesterone (50–2000 mg.). The progesterone was given parenterally, orally, buccally and vaginally. Endometrial biopsies were performed before and during therapy. In 70 trials the best response was obtained with aqueous parenteral progesterone, but this method was accompanied by severe local reaction. Progesterone in oil was about one half as effective. Sublingual and buccal routes required 5 times as much as the parenteral. The oral route needed 20 times as much to produce the same endometrial picture. Preliminary trials indicated that the vaginal route gave a somewhat better response than did the buccal. Escape bleeding originally took place in nearly

50 per cent of the cycles, irrespective of the amount or manner of administration of progesterone. This was corrected by increasing the estrogen every third day. A true decidual response was never noted. Bleeding took place uniformly from twenty-four to sixty hours after discontinuation of therapy, irrespective of the type of endometrial response. Basal body temperatures, which were taken in all cases, were shown to be a poor index of progestational activity as judged by the endometrial pattern.

110. Effect of Estrogen and Testosterone on Rate of Nucleic-Acid Synthesis in Mouse Mammary Cancer *In Vivo*.

Alois Vasicka, M.D. and *Somers H. Sturgis, M.D.** Department of Surgery, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

We have previously reported the effect of estrogens and androgens on the *in vitro* rate of nucleic-acid synthesis in mouse mammary cancer. (*Am. Coll. Surg. Forum* Nov. 16, 1954). The present report records the results of comparable experiments using an *in vivo* technique. A strain of C_3H mice bearing spontaneous tumors in the female and grafted tumors in the male was used. All animals were castrated. One week later, standard hormone injections were given for three days before sacrifice. Twenty-four hours before sacrifice, 25 μc. of P^{32} as phosphate was given intraperitoneally. The tumor tissue was pooled after necropsy. Total phosphorus and radioactivity were assayed on aliquots in order to calculate the specific activity of steroid-treated tumors in contrast to untreated castrated controls. The effects of estrogens and androgens on this specific activity of tumors from both males and females are reported. It is believed that this study may throw some light on studies now under way in this laboratory concerning the effect of sex steroids on nucleic-acid synthesis in human mammary cancer.

ADRENALS AND ADRENOCORTICAL STEROIDS

111. Hazard of Corticotropin and Cortisone in Patients with Hypercholesteremia.

*David Adlersberg, M.D.,** *Jenny Stricker, M.D.* and *Hillard Himes, M.D.* Mount Sinai Hospital, New York, N. Y.

Thrombo-embolic phenomena are of great practical importance as possible complications of the use of corticotropin and cortisone. These complications were observed in 4 patients with hypercholesteremia, idiopathic or secondary to other disease, leading to the impression of a casual relationship between the pre-existing elevation of serum cholesterol concentration and the thrombo-embolic phenomena. Patient 1, a 52-year-old white female, had an attack of acute coronary insufficiency shortly after initiation of corticotropin therapy for a chronic form of diarrhea of unknown origin. She proved to have hypercholesteremia and was a member of a family with this abnormality. In Patient 2, a 34-year-old male, pulmonary thromboses developed following use of cortisone for rheumatoid arthritis. This man also proved to have hypercholesteremia and to be a member of a hypercholesteremic family. In Patient 3, a 40-year-old white male who had a previous coronary occlusion, severe chest pain, fever and pleural effusion developed following the use of hydrocortisone for the treatment of gout. These phenomena were interpreted as being due to a pulmonary infarction. He and some of his siblings had hypercholesteremia. In Patient 4, a 52-year-old male, a recurrent myocardial infarction developed while he was undergoing treatment with cortisone for the nephrotic stage of chronic glomerular nephritis associated with marked hypercholesteremia.

112. Effect of Age on the Steroid Output by Rat Adrenal Glands *In Vitro*.

Marion K. Birmingham, Ph.D., Marie-Louise Desbarats, M.Sc. and *M. M. Hoffman, M.D.** Allan Memorial Institute, McGill University, Montreal, Canada

The steroid outputs *in vitro* by rat adrenal glands (Elliott *et al.*: *Endocrinology* **55**: 721, 1954) from animals aged less than 2 months, 4–8 months and over 24 months were compared in the presence and absence of added ACTH. There was no significant difference in the output by unstimulated adrenals from the 2 older age groups, nor was there any difference in their response to ACTH. Adrenals from rats under 2 months of age produced less total steroid than adrenals from the older age groups. The production per unit of adrenal weight by the youngest group was slightly less than that of the older groups. However, the glands from the youngest group maintained a constant rate of steroid production over three hours, whereas the rate in the 2 older groups decreased significantly with time. The response to ACTH, when expressed in steroids produced per 100 mg. of adrenal, or per unit of body surface, did not differ significantly from that of the older animals. However, the steroid production per unit of body weight was nearly twice as high in the youngest group as in the older groups, both in the presence and absence of added ACTH.

113. The Production of "Steroid Diabetes," Albuminuria and Glomerular Changes Simulating Diabetic Glomerulosclerosis by Long-Term Administration of Adrenocortical Steroids.

*J. M. B. Bloodworth, Jr., M.D.** and *George J. Hamwi, M.D.** Department of Pathology and Division of Endocrinology, Department of Medicine, Ohio State University College of Medicine, Columbus, Ohio

Rich reported the incidental finding of nodular glomerular lesions and albumin in alloxan-diabetic rabbits receiving cortisone, and pointed out their superficial morphologic similarity to the lesions of human diabetic glomerulosclerosis. Previous studies in this laboratory showed that the experimental lesions represent thrombi in dilated capillary loops (micro-aneurysms). The thrombi were composed of PAS-positive material (mucopolysaccharide) and lipid. They resembled the "hyaline-fibrinoid" lesions frequently seen in the kidney of human diabetic patients. Similar lesions could also be produced in normal rabbits by the administration of cortisone or hydrocortisone for three weeks. The administration of DCA, ACTH, co mpound A, or whole Lipo-adrenal cortical extract did not produce lesions. The administra tion of ACTH and DCA to alloxan-diabetic rabbits was also unsuccessful. Cortisone in white rats failed to produce lesions in three weeks. Long-term studies have been carried out with cortisone and hydrocortisone in normal and alloxan-diabetic rabbits in an attempt to produce typical nodular lesions of diabetic glomerulosclerosis. These studies have shown a progressive diffuse thickening of the axial fibers of the glomerulus with a pattern quite similar to that described by Bell in human diabetes mellitus under the name "diffuse glomerulosclerosis."' Occasional lesions have shown a nodularity.

114. Isolation of Certain Metabolites Following Perfusion of Cortisol through Isolated Rat Liver.

E. Caspi, Ph.D. and *O. Hechter, Ph.D.** Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

In continuation of previous investigations designed to chart the metabolic transfor-

mations of corticosteroids in isolated rat liver (Caspi, E. and Hechter, O.: *Arch. Biochem. & Biophys.* **52**: 478, 1954) the perfusion products obtained from radioactive cortisol have been studied. After the perfusion of 200 mg. of cortisol-4-C^{14} (20,000 cts. per min. per mg.) in 2 liters of citrated bovine blood through 2 rat livers for 6.4 circulations, the following products have been isolated from the perfusate: a) allopregnane-3β,11β,17α, 20β,21-pentol (38.9 per cent), b) allopregnane-3β,11β,17α,21-tetrol-20-one (19.3 per cent), c) Δ^4-pregnene-11β,17α,20β,21-tetrol-3-one (15.0 per cent), d) androstane-3β,11β-diol-17-one (2.0 per cent), e) Δ^4-androstene-11β-ol-3,17-dione (9.4 per cent). The foregoing metabolites were obtained as crystalline products after systematic paper chromatographic fractionation, using methods previously described. They were definitely identified by comparison of infrared spectra and other physical data with appropriate authentic samples. The specific activity of the products isolated was similar to that of the cortisol used; the approximate yields of the products, shown in parenthesis, are calculated from the counts observed in the various zones of the papergrams. Approximately 16.4 per cent of the radioactivity, which appeared to represent a minimum of 17 substances, could not be identified. The results presented are in full agreement with the previously suggested sequence for the metabolism of cortisone in isolated rat liver, in that the observed reactions demonstrate oxidative cleavage of the cortisol side chain, associated with reductions of carbonyl, but not hydroxyl, groupings.

115. The Effect of Hydrocortisone and Ascorbic Acid on Adrenal Function in the Cold-Exposed Guinea Pig.

S. A. D'Angelo, Ph.D., C. E. Stevens, Ph.D., K. E. Paschkis, M.D.** and *A. Cantarow, M.D.** Jefferson Medical College, Philadelphia, Pa.

Adult female guinea pigs exposed to lowered environmental temperatures (7° ±2° C.) for six to seven weeks showed significant increases in adrenal size and urinary corticoid excretion while maintaining normal ascorbic-acid concentrations in the adrenals. The administration of hydrocortisone acetate (12.5 mg. daily, subcutaneously, for forty-six days) completely inhibited adrenal hypertrophy and significantly reduced adrenal ascorbic-acid concentration in the cold-exposed animal. High-level dosage with sodium ascorbate (100 mg. daily, intraperitoneally, for forty-six days) increased ascorbic-acid concentration in the adrenal but failed to prevent the hypertrophy to cold. Determinations of corticoids in urines of animals after one, three, five and seven weeks of exposure revealed substantial increases in corticoid excretion from control values obtained at 23° ±2° C. The mean corticoid excretions over the entire exposure period, calculated as compound F excreted per twenty-four hours per animal, were 299 μg., 379 μg., and 921 μg., respectively, for the saline, ascorbic-acid and hydrocortisone-treated groups, as against 140 μg. in room-temperature controls. The results are believed to signifiy that augmented adrenal function in cold stress can be reduced by high-level dosage with adrenocortical steroids but not with ascorbic acid. The effect is presumed to be mediated by corresponding reduction in the activity of the ACTH mechanism in the adenohypophysis.

116. Effect of Roentgen Irradiation of the Pituitary Region in Cushing's Syndrome without Adrenocortical Tumor.

F. C. Dohan, M.D., A. Raventos, M.D., N. Boucot, M.D.* and *E. Rose, M.D.** Hospital of the University of Pennsylvania and School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Roentgen irradiation to the pituitary region of 12 patients (1932–1951) with Cushing's syndrome (without demonstrable adrenocortical tumor) was followed in 5 instances by "complete" remission. The mean times (in months) for marked improvement were: fading of purple striae, 11; acne, 7; bodily and facial configuration, 7; psyche, 6; return of menses, 6; and diastolic pressure less than 85 mm. Hg., 4. In the other 7 patients, the response was unsatisfactory, although 3 had a partial remission but with continuing high blood pressure and other signs. The mean total tissue roentgen dose in the satisfactory group was 4350 (range 3800–5250) and in the unsatisfactory group 1339 (range 400–4550). The mean tissue roentgen dose per day was 113 (range 101–129) for the satisfactory group and 62 (range 10–85) for the unsatisfactory. Analysis of these data plus study of the available literature suggests that the total number of days over which the total dosage is delivered is an important factor in effectiveness of therapy.

117. Effect of Cortisone and High Cholesterol Diet on Lipid Components and Phospholipid Turnover of Plasma, Liver and Aorta in Rabbits.

*Abraham Dury, Ph.D.** Dorn Laboratory Medical Research, Bradford Hospital, Bradford, Pa.

A pellet chow supplemented with 1 per cent cholesterol and 2.8 per cent vegetable fat was given to a group of rabbits for thirty-five days. Six received 10 mg. of cortisone intramuscularly daily and 7 received physiologic saline daily. Another group was given a pellet chow supplemented only with the vegetable fat. On the final day 6 rabbits of each group received 0.5 millicurie of P^{32} intravenously and were sacrified six hours later. The total cholesterol concentrations were significantly elevated (mainly ester cholesterol) in liver, plasma and aorta of the cholesterol-fed animals; liver phospholipid concentration was depressed; and the plasma phospholipid level was markedly elevated. Aortic phospholipid and neutral fat in liver, plasma and aorta of the cholesterol-fed rabbits were unaffected. The turnover of liver and plasma phospholipid was significantly increased in the former group but the aortic phospholipid specific activity did not change. Cortisone in cholesterol-fed rabbits induced 300 per cent and 500 per cent increases in liver and plasma neutral fat, respectively, but all aortic lipid fractions and the phospholipid and cholesterol in liver and plasma did not change. The turnover of phospholipid in liver and aorta was not influenced by cortisone but the amount of radioactive phospholipid per cc. of plasma was increased 200 per cent. The results show that phospholipid metabolism was significantly altered in rabbits fed a high-cholesterol diet. Cortisone in this type of animal mainly influenced the mobilization and transport of neutral fat but also affected the turnover of plasma phospholipids.

118. Study of Adrenal-Pituitary and Sympathico-Adrenal Systems in Psychomotor Stress and after ACTH Injection.

Fred Elmadjian, Ph.D., Hudson Hoagland, Ph.D., Eric Bloch, Ph.D., J. R. Bergen, Ph.D. and *Gregory Pincus, Sc.D.** Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and Worcester State Hospital, Worcester, Mass.

The excretions of 17-KS, Na, K, adrenaline (A) and noradrenaline (NA) as well as eosinophil counts were determined in subjects (aged 16–20) undergoing psychomotor stress on the Hoagland-Werthessen pursuitmeter, and after ACTH injection (Zinc Organon). Under conditions of the stress experiments, which included anoxia, the 17-KS output did not increase but there was a clear decline in the eosinophil count and

an increase in A excretion. The NA excretion increased, but not consistently. Two features of these data are worth noting: 1) Under similar conditions of pursuitmeter stress we had previously found increases in 17-KS excretion. However these previous experiments had been conducted on young men over 20 years of age. It appears that though there were objective signs of stress in the younger men in the present study there was no increased excretion of 17-KS following stress. 2) The eosinophil count decreased in all cases and the data indicate that eosinophil counts are related more to A excretion than to adrenocortical function as measured by urinary 17-KS. ACTH injections for three consecutive days at 20 I.U./day, given to 4 men, induced a marked increase in urinary 17-KS and an elevated K/Na ratio of the urine with a decrease in the NA excretion rate. The A excretion did not seem to be affected by the ACTH. Upon cessation of the ACTH injection there was a return of the 17-KS to normal with a pronounced drop in the K/Na ratio and a return of the NA excretion to normal levels.

119. Evaluation of Adrenocortical Function by Measurement of the 17-Hydroxycorticosteroid Levels in the Blood and Urine.

Edwin Englert, Jr., M.D., Harold Brown, M.D., Donald G. Willardson, A. B.* and *E. L. Simons, M.A.* Veterans Administration Hospital, and the Departments of Medicine and Biochemistry, University of Utah College of Medicine, Salt Lake City, Utah

Measurement of the 17-hydroxycorticosteroid (17-OH-CS) levels in plasma by the modified technique of Nelson and Samuels, and in urine by a modification of the method of Reddy, Jenkins and Thorn has proved to be an accurate means for evaluating the various types of adrenocortical hypo- and hyperfunction. By observing the effects of a standard dose of ACTH, it was possible to separate the cases of hypofunction into absolute and relative categories, and those of hyperfunction into benign and malignant groups. In patients with true Addison's disease, there were very low, or zero 17-OH-CS levels in the plasma and these did not increase with ACTH stimulation. In individuals with relative adrenocortical insufficiency, the plasma 17-OH-CS levels were normal or decreased and these also did not rise with ACTH stimulation. In patients with Cushing's syndrome, plasma 17-OH-CS levels were elevated and responded in an exaggerated fashion to ACTH stimulation if the syndrome were due to hyperplasia or adenoma. In cases of carcinoma, no change was observed after ACTH. The 17-hydroxycorticoid excretion in the urine reflected the plasma levels.

120. Microchemical Estimation of Aldosterone in Urine.

A. G. Gornall, Ph.D., C. Gwilliam, B.Sc.* and *A. E. Hall.* Department of Pathological Chemistry, University of Toronto, Toronto, Canada

Although the "salt-retaining factor" in urine can be assayed biologically on relatively crude extracts it may not be correct to assume that the effect is always due mainly to aldosterone. Estimations of the aldosterone content of urine have been made by the following procedure: Four-day collections of urine were adjusted to pH 2 and cycled intermittently in a continuous extractor through successive changes of methylene chloride over a 24-hour period. The total volume of solvent, equal to the volume of urine, was washed with 1/10 volume of alkali and water and then removed in a flash-evaporator. The residue was carried through a benzene:water transfer, the aqueous phase taken to dryness and the residue dissolved in methanol. The extract was chro-

matographed first in a petroleum ether/propylene glycol:water (50:50) system. The starting zone was eluted and the steroids separated by running them successively in the following systems: (a) benzene/methanol:water (Bush), (b) benzene:10 per cent-ethyl acetate/methanol:water (Bush), (c) toluene/propylene glycol (Zaffaroni) and repeat (b). This effected the separation of aldosterone in a high state of purity. Quantities were measured by ultraviolet absorption and DNPH reaction, the latter more sensitive and characteristic for aldosterone.

121. Resistance of Mice to Brucella Somatic Antigen; 24-Hour Periodicity and the Adrenals.

Franz Halberg, M.D., Wesley W. Spink, M.D., Paul Albrecht, B.S.* and *Raymond J. Gully, M.D.* University of Minnesota Medical School, Minneapolis, and Cambridge State School and Hospital, Cambridge, Minnesota.

CBC mice were singly housed, fed *ad lib.* and kept in light from 06:00 to 18:00 and in darkness from 18:00 to 06:00. They were injected intraperitoneally with Brucella somatic antigen (endotoxin) by day or by night. The night phase of the daily cycle, known to be associated with adrenal activation in mice (*J. Lancet* **73**: 20, 1953), was also associated with enhanced resistance to endotoxin, if low lethal doses were employed. The mortality rate of intact mice given identical doses of endotoxin was 29 per cent by day ($N=226$) and 19 per cent by night ($N=281$). Mice succumbing to day injections lived for 37.1 ± 7.3 hours after the injection; those succumbing to night injections lived for 75.6 ± 15.0 hours (P 0.05). Consistently, hypothermia preceded by several hours death from endotoxin. In adrenalectomized mice, this hypothermia was induced by lower doses of endotoxin and occurred earlier. The ability of corticoids to protect against the hypothermia induced by 0.2 mg. of endotoxin in adrenalectomized mice was used for assaying antitoxic potency. In such assays of DCA, aldosterone, cortisone acetate and 9-fluorohydrocortisone acetate, the latter was the most active compound.

122. Adrenocortical and Psychologic Response to Stress in Man.

S. Richardson Hill, Jr., M.D., Frederick C. Goetz, M.D.,* Henry M. Fox, M.D., Benjamin Murawski, Ph.D., Seymour J. Gray, M.D., L. Krakauer, M.D.* and *George W. Thorn, M.D.** Department of Medicine, Harvard Medical School and Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

This study was designed to clarify the role of the adrenal cortex and various psychologic factors in man's response to stress. The 9 subjects were members of the 1953 and 1954 college varsity crew, studied during the final two weeks of preparation for a four-mile race. Eosinophil counts were made at 9 A.M., 5 P.M., and 9 P.M. Four-hour urine specimens were collected from 9 A.M. to 9 P.M. and one specimen from 9 P.M. to 9 A.M. for determination of the 17-hydroxycorticoid, 17-ketosteroid and uropepsin excretion. Psychologic measurements included two Rorschach tests, thematic apperception tests and personal interviews. The eosinophil response showed a mean 20 per cent increase on the control day, a 30 per cent decrease on the regular practice day, a 69 per cent decrease on the time-trial day and a 70 per cent decrease on the race day. The steroid data revealed highly significant ($P<.01$) differences between the values on race day as compared to those on control and practice days, with no significant difference between values on the control day as compared with those on practice day. On race day there was an obliteration ($P<.01$) of the daily diurnal decrease in 17-hydroxycorticoids.

123. Biliary Excretion of Radioactivity After Hydrocortisone-4-C^{14} Administration to Rats.

Paul M. Hyde, Ph.D. and *Robert H. Williams, M.D.** University of Washington School of Medicine, Seattle, Wash.

The biliary route has been shown to be the major pathway of excretion of radioactivity after isotopic compound F is given to rodents with the common bile duct cannulated. The effects of the various routes of administration on the excretion of biliary C^{14} were determined. Accordingly, a dose of 50 micrograms of hydrocortisone-4-C^{14} was given intravenously, intramuscularly, sublingually, or intragastrically to male rats with the common bile duct cannulated. The average amounts of radiocarbon excreted in the bile in the first four-hour period, for each group of 6 or more animals, were 84, 76, 52 and 30 per cent after intravenous, intramuscular, sublingual, and intragastric administration, respectively. Similarly, the averages of the total C^{14} content found in bile for a 48-hour period were 90, 84, 80 and 76 per cent, respectively. In each series, when bile from one animal with a biliary fistula was introduced intraduodenally into other rats with biliary fistulas, about 35 per cent of the radioactivity was unabsorbed from the intestinal tract. Approximately 60 per cent of the C^{14} readministered duodenally to each group was excreted in the bile over a 36-hour period, indicating marked enterohepatic circulation of hydrocortisone-4-C^{14} or its radiometabolites.

124. Intravenous Administration of Aldosterone to the Adrenalectomized Rat As a Method of Assay.

Julia Lobotsky, M.S., JoAnne Buss Hannye, M.A. and *Charles W. Lloyd, M.D.** State University of New York Upstate Medical Center, Syracuse, N. Y.

A polyethylene cannula was placed in the right jugular vein of male, young adult, Sprague-Dawley rats. Several days later bilateral adrenalectomy was performed. Forty-eight hours after adrenalectomy the animals were used for assay of aldosterone. Usually the animals could be used for assays on three consecutive days. At zero hours, 4 ml. of distilled water was injected intraperitoneally. The salt-retaining material was given in three divided doses into the cannula at zero, one and two hours. Urine was collected for four and a half hours, and sodium to potassium ratios calculated. This method appeared to have more than twice the sensitivity of that described by Kagawa, Shipley and Meyer (*Proc. Soc. Exper. Biol. & Med.* **80**: 281, 1952). Salt-retaining activity could be detected in chloroform extracts of 25 ml. of plasma of some presumably normal subjects and in as little as 1 ml. of plasma of cirrhotic patients. In two preliminary studies hydrolysis of plasma steroids with β-glucuronidase caused a marked increase in 17-OH corticosteroids with no increase in salt-retaining material.

125. Effects of Hydrocortisone Topically Applied on Keloids and Hypertrophic Scars.

R. E. Mancini, M.D. and *S. Stringa, M.D.* (introduced by F. A. de la Balze, M.D.). Instituto Nacional de Endocrinologia, Buenos Aires, Argentina

Six cases of flat keloids and 6 cases of hypertrophic scars were treated topically with an ointment containing 1 per cent of hydrocortisone acetate, three times daily during a number of months. A histologic and histochemical study of the changes was carried out by means of biopsies before, and at three and six months after the treatment. It was observed: 1) Itching disappeared in keloids after the first week; congestion decreased after two months, and there was atrophy of the keloid at the end of four to

six months. 2) The biopsies showed a progressive decrease in the hyperplasia of juvenile fibroblasts, collagenous fibers, interfibrillar mucopolysaccharide and vascularization. 3) Finally there was atrophy in most of the fibroblasts, with decrease in their cytoplasmic ribonucleoprotein and glycoprotein content. The collagenous fibers showed tumefaction, slight basophilia and partial disintegration. Elastic fibers were completely fragmented and the basement membrane reduced. 4) The cutaneous epithelium and the mast cells showed no changes. 5). In hypertrophic scars, the changes observed were minimal. 6) The biopsies carried out in keloids and hypertrophic scars during treatment did not cause abnormal scars. 7) Other cases, treated with the vehicle of the hormone as controls, did not undergo any changes.

126. Corticosteroid Secretion in the Adrenal Vein of Dogs Exposed to Cold.

Don H. Nelson, M.D., Richard H. Egdahl, M.D.* and *David M. Hume, M.D.* Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

The adrenal vein in 11 male dogs was cannulated by the procedure described by Hume and Nelson (*Fed. Proc.* **13**: 73, 1954). Samples were obtained for the determination of 17-hydroxycorticosteroids secreted during operation and for two to ten days after the cannula had been put in place. The animals were allowed to recover from the operation, placed in a cold room at −10° C., and minute flows of adrenal venous blood collected at intervals during the exposure to cold and during control periods at room temperature. In order to test the ability of the animal's adrenal to respond to stimulation, ACTH was administered intravenously on a number of occasions. The 17-hydroxycorticosteroid secretion was elevated during and immediately following operation but fell to near zero levels by the third day in 8 of 11 animals. Administration of ACTH increased the level markedly but exposure to temperatures of −10° C. for periods up to thirty-three hours caused no statistically significant rise in the level of 17-hydroxycorticosteroids. Exposure to more severe cold under anesthesia, to the extent of producing hypothermia, caused a definite fall in corticosteroid secretion even when the animals were subjected to severe surgical stress or given intravenous infusions of ACTH. Warming of the dogs following cold exposure generally produced an increased secretion of 17-hydroxycorticosteroids from the adrenal.

127. The Chromogenic Spectra of Steroids in 100 Per Cent Phosphoric Acid.

Wojciech Nowaczynski, D.Sc. and *Paul Steyermark, Ph.D.* (introduced by Eleanor Venning, Ph.D.).* Clinical Research Department, Hotel-Dieu Hospital, Montreal, Canada

A new method was developed for the identification of steroids. Dry steroids were heated fifteen minutes at 105° C. with 100 per cent phosphoric acid (70 mg. of phosphoric anhydride added to 59 ml. of 85 per cent phosphoric acid, density 1.7). The spectra were determined with a Beckman spectrophotometer within the range of 220 to 600 mμ, against a similarly treated blank of 100 per cent phosphoric acid. Previous heating of the acid to 100° C. was necessary to decrease its density and to make the transfers easier. All curves were checked over a 24-hour period, with two additional ten-minute heating periods and they were found to be perfectly stable. The optical densities ranged from 0.8 to 2.0 for the steroid concentration of 25 micrograms/ml. This procedure was applied to the following steroids: aldosterone, cortisone, hydrocortisone, corticosterone, 11-desoxycortisone, DOC, 11-dehydrocorticosterone, 17α,21-dihydroxy-

pregnane-3,11,20-trione, 3α,17α,21-trihydroxypregnane-11,20-dione, 11β,17α,21-trihydroxypregnane-3,20-dione, 3α,11β,17α,21-tetrahydroxypregnane-20-one, 11α,17β,21-trihydroxy-Δ4-pregnene-3,20-dione, 17α-hydroxy-Δ4-pregnene-3, 20-dione, androsterone, and dehydroisoandrosterone. All the spectra were different from those obtained with concentrated sulfuric acid and, in nearly all cases, characteristic of each compound. There was a straight-line relationship between the concentration of steroids and the absorptivity.

128. Blockade of Stress-Induced Adrenal Ascorbic-Acid Depletion.

*E. A. Ohler, Ph.D.** and *R. W. Sevy, M.D.** Departments of Physiology and Pharmacology, Temple University School of Medicine, Philadelphia, Pa.

Adrenal cortical extract (ACE), morphine and dibenzyline were tested in rats for the capacity to prevent adrenal ascorbic-acid depletion caused by unilateral adrenalectomy, sham adrenalectomy and epinephrine. Adrenal ascorbic-acid depletion following unilateral adrenalectomy or sham adrenalectomy was almost completely blocked by morphine (2 mg./100 Gm. subcutaneously) ($p = <.01$; $<.01$), partially blocked by ACE (1.0−1.5 cc./100 Gm.) ($p = <.05$; $<.01$), and totally uninfluenced by dibenzyline (0.3 mg./100 Gm. i.v.) ($p = >0.4$; >0.1). Adrenal ascorbic-acid depletion following epinephrine (0.025−0.06 mg./100 Gm.) was partially blocked by all three agents ($p = <.01$ in each case). On the basis of these and previous results it appears improbable that endogenous epinephrine plays a primary role in the pituitary-adrenal response to stress. On the other hand the data are interpreted to support the contention that utilization of adrenal corticoids (with temporary reduction in plasma levels) is a basic factor in the regulation of ACTH secretion. Morphine may act to block the response of pituitary or hypothalamic receptors to lowered plasma corticoid levels.

129. The Effect of Administered Corticosteroids on Adrenocortical Steroidogenesis.

Richard Orr, M.D., Vincent Di Raimondo, M.D., Mary Flanagan, M.D. and *Peter H. Forsham, M.D.** Metabolic Unit of Research in Arthritis and Allied Diseases, University of California School of Medicine, San Francisco, Calif.

In adrenalectomized patients approximately 25 per cent of administered hydrocortisone (F) appears in the urine as butanol-extractable Porter-Silber chromogens as determined by the methods used in this study. Approximately 5 per cent is recovered as neutral 17-ketosteroids (17-KS). The same holds for fluorohydrocortisone (FF) with respect to 17-OHC. Metacortandracin (MC) appears to yield higher values. Using these recovery values to correct for the contribution from exogenously administered steroid, the urinary 17-OHC and 17-KS derived from the adrenal were found to decrease progressively in normal subjects given increasing doses of these steroids. Endogenous production was found to cease when 30 to 50 mg. of F was administered daily, suggesting this as the normal adrenocortical output. Since the pituitary suppressant action of F, FF and MC, determined in this manner, closely correlates with their reported anti-inflammatory activity, a convenient means of assay of antiphlogistic properties is suggested. A simple test was developed to assess the integrity of the pituitary control of adrenocortical activity, utilizing the ACTH suppressant action of orally administered FF. The amount of FF used is the biologic equivalent of 4 times the 24-hour output of 17-OHC.

130. Sequence of Effects of Cortisone, Hydrocortisone and Desoxycorticosterone Acetate Upon Urinary Sodium, Potassium, Chloride and Volume.

S. Pearson, Ph.D., H. Reckendorf, M.D.* and *T. H. McGavack, M.D.** New York Medical College, Metropolitan Hospital, Research Unit, Welfare Island, New York, N. Y.

A 40-year-old female with rheumatoid arthritis was given intravenous infusions of 1000 ml. of normal saline solution from 9:00 A.M. to 5:00 P.M. for fifteen days. On twelve days, different amounts of cortisone, hydrocortisone, desoxycorticosterone acetate, or a combination of two of these steroids were incorporated into the saline from 1:00 P.M. to 5:00 P.M. Urine was collected hourly from 9:00 A.M. to 1:00 P.M., and at 5:00 P.M. 9:00 P.M. and 9:00 A.M. Urinary volume, sodium, potassium and chloride were measured. After infusion of steroids, urinary excretion and concentration of sodium and chloride, and urinary volume decreased. The excretion and concentration of potassium increased. Significant changes were observed first in the concentration of potassium. The excretion of potassium, the excretion and concentration of sodium and chloride, and urinary volume were next affected in the order given. Eight to twenty hours after the infusion of steroids was begun, the urinary excretion and concentration of all substances studied were still altered, with the exception of potassium excretion. On eight different days measurements were made of the levels of sodium, potassium, chloride and sugar in blood at 9:00 A.M., 1:00 P.M., and 5:00 P.M. of the day of infusion, and 9:00 A.M. the day after the infusion. No changes were observed.

131. Effect of Intravenous Infusion of Hydrocortisone and Derivatives Upon Carbohydrate Metabolism in Man.

Murry Persky, M.D., Joseph Linsk, M.D., Marian Isaacs, M.D., John P. Jenkins, M.D., Milton Rosenbluth, M.D. and *Herbert S. Kupperman, M.D.** Department of Therapeutics, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Hydrocortisone and derivatives have been administered intravenously by infusion alone and in combination with glucose or insulin to a series of patients with decreased and normal glucose tolerance. During the infusion of the steroids, blood glucose levels were determined. When glucose tolerance tests were performed simultaneously during the hydrocortisone infusion the glucose was administered at the beginning of the infusion. Insulin tolerance tests were performed during the infusion of hydrocortisone, after a peak response to hydrocortisone had been achieved. Changes in blood eosinophils during the infusion with hydrocortisone were noted and indicated that a significant fall occurred within two hours of the infusion. Blood phosphorus levels and urinary phosphorus excretion were determined in several selected instances. The infusion of hydrocortisone and derivatives induced a decrease in glucose tolerance in patients with both normal and decreased tolerance. However, administration of insulin during the hydrocortisone infusion resulted in normal insulin responsiveness, comparable to that noted in patients not receiving hydrocortisone. Hydrocortisone did not interfere with glucose utilization, as manifested by the drop in blood phosphorus concentration noted in these patients.

132. The Determination of Total 17-Hydroxycorticosteroids in Plasma.

William J. Reddy, A.B., Najib Abu Haydar, M.D.* and *John C. Laidlaw, M.D.** Department of Medicine, Harvard Medical School and the Medical Service, Peter Bent Brigham Hospital, Boston, Mass.

A method has been devised for the estimation of plasma 17-hydroxycorticosteroids. The procedure consists in the precipitation of plasma proteins by the Somogyi technic with butanol extraction of the steroids from the protein-free solution in a manner similar to that described for urine (*Metabolism* **3**: 489, 1954). Colorimetry can then be carried out without previous hydrolysis and chromatography. This technic gives low blank values, requires little time, and permits measurement of total 17-hydroxycorticoids, including the conjugated fraction. The desirability of measuring both free and conjugated 17-hydroxycorticoids is evident. Chromatography of the conjugated fraction after hydrolysis indicates that tetrahydrohydrocortisone is its major constituent. In normal adults the concentration of total 17-hydroxycorticoids was 30–80 micrograms per 100 ml. of plasma. The concentration of conjugated 17-hydroxycorticosteroids was greater than that of the free form, although the proportion of conjugated to free in plasma was not as high as that usually found in urine. The diurnal variation found in urinary steroid excretion was also observed in the blood steroids.

133. Quantitative Assay of 11-β-Hydroxylase Activity in Whole Adrenal Homogenates.

*Betty L. Rubin, Ph.D.** and *Ralph I. Dorfman, Ph.D.** Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

Using an adaptation of the 11-β-hydroxylating system of Hayano and Dorfman (*J. Biol. Chem.* **201**: 175, 1953), conditions were standardized for the quantitative assay of 11-β-hydroxylase activity in whole adrenal homogenates. A 50 per cent homogenate was prepared in a Virtis homogenizer and serial dilutions made. Of each homogenate concentration, 1.5 ml. was incubated with an excess of substrate (usually 1–1.5 mg. of 11-desoxycortisol). The reaction mixture was extracted with ethyl acetate and the dried ethyl-acetate extract chromatographed in the toluene-propylene glycol system for twenty to twenty-four hours on a 2-cm. strip. Cortisone and cortisol were run as standards on adjacent strips. The blue tetrazolium reaction was carried out on 2-mm. strips cut from each edge of the 2-cm. strip, and the zones corresponding to cortisone and cortisol eluted and determined by absorption at 240 mμ. Micrograms of 11-oxygenated products obtained were plotted against the logarithm of the milligrams of nitrogen in 1.5 ml. of the various tissue dilutions. A straight-line relationship was found over the range from 1 to 10 mg. of N. The assay method was standardized, using cow adrenal tissue and was applied to normal adrenal tissue from bulls, pigs and rats, and to diseased adrenal tissue from human subjects.

134. Some Psychologic and Biochemical Relationships in Healthy Subjects Receiving Intravenous ACTH on Successive Occasions.

Melvin Sabshin, M.D., Harold Persky, Ph.D. and *Francis A. Board, M.D.* (Introduced by Clarence Cohn, M.D.). Institute for Psychosomatic and Psychiatric Research and Training, Michael Reese Hospital, Chicago, Ill.

College students were given three doses of ACTH (5, 10 and 15 units) intravenously over a four-hour period on successive days. The psychologic status of each subject was determined by a diagnostic psychiatric interview, and the psychologic response by pre- and post-ACTH interviews and subject diaries. Most subjects achieved relatively high levels of anxiety on the early portion of the first day of testing but neither the initial level nor the plasma 17-hydroxycorticosterone concentration (17-OHCS) was of the magnitude found in a group of psychiatric in-patients. The initial anxiety and plasma 17-OHCS level was not significantly correlated with the response of either variable to

ACTH. The increase in plasma 17-OHCS concentration following ACTH was not accompanied by an increase in anxiety or other affective response. The 17-OHCS response to 5 units of ACTH was not statistically different from the response to 15 units. Randomization of dose administration in another group of students did not result in differential 17-OHCS response between 5 and 15 units of ACTH, indicating that the biochemical response was not a simple function of the increased anxiety on the first day of the procedure. The study indicated that administration of tolerance doses of ACTH to relatively healthy subjects does not produce deleterious psychologic effects.

135. A Defect of 17-Hydroxycorticosteroid (17-OHCS) Metabolism in Dying Patients.

Avery A. Sandberg, M.D., Kristen Eik-Nes, M.D. and *Claude Migeon, M.D.** Departments of Medicine and Biochemistry, University of Utah College of Medicine, Salt Lake City, Utah.

This study was undertaken to elucidate further the causes of high concentrations of plasma 17-OHCS in the agonal state. Following injection of nonradioactive hydrocortisone or 4-C^{14}-hydrocortisone, the concentration of free steroid in plasma of dying patients fell more rapidly for sixty minutes than in normal subjects. Thereafter, concentrations of free hydrocortisone in the plasma of moribund patients were maintained at a high plateau, contrasted to rapidly falling levels in normal individuals. Concentrations of conjugated 4-C^{14}-hydrocortisone were lower in dying patients than in normal subjects. Apparently, the initial rapid fall in concentration of hydrocortisone in dying patients is due to abnormal diffusion from the plasma. Following equilibration, high levels were due to impaired metabolism. Urinary 17-OHCS excretion in dying patients was not increased, despite elevated plasma levels, and showed a normal ratio of free to conjugated steroids. Following 4-C^{14}-hydrocortisone administration 25–30 per cent appeared in the urine in forty-eight hours (normal 90 per cent). In dying patients plasma clearance of administered tetrahydrocortisone was comparable to that in normal subjects. Inability to clear unreduced administered steroid (hydrocortisone) suggests that inability to hydrogenate 17-OHCS, rather than failure to conjugate, is a major defect in the metabolism of these compounds in dying patients.

136. Importance of Glucose in the Stimulation of Isolated Rat Adrenals by ACTH.

E. Schonbaum, Ph.D., Marion K. Birmingham, Ph.D. and *M. Saffran, Ph.D.* (introduced by R. A. Cleghorn, M.D.). Allan Memorial Institute, McGill University, Montreal, Canada.

Glucose is required for the optimal stimulation by ACTH of the formation of corticoids by rat adrenals *in vitro*. The importance of glucose was confirmed by studies of the effects of inhibitors on the formation of corticoids. Inhibitors of the Krebs cycle (malonate, *trans*-aconitate and fluoroacetate) were *without* effect on ACTH stimulation, whereas inhibitors of steps in the conversion of glucose to acetyl CoA (iodoacetate, fluoride, and arsenite) inhibited strongly. Azide and dinitrophenol, which uncouple phosphorylations from oxidations, also inhibited the effect of ACTH. Anaerobiosis inhibited the production of corticoids in the presence and absence of added ACTH. These findings imply that a fully-functioning glycolytic system is required for the activation of the adrenal cortex by ACTH, and that the Krebs cycle reactions are not directly involved.

137. Interactions Between Corticoid and Folliculoid Hormones in the Regulation of Anabolism, Lymphatic Tissue and Inflammation.

*Hans Selye, M.D.** Institut de Médicine et de Chirurgie expérimentales, Université de Montréal, Montréal, Canada.

The effects of 5 to 200 μg./day of estradiol upon anabolism (body-weight), lymphatic tissue (thymus, spleen) and inflammation (exudate and granuloma-formation in the "granuloma-pouch") were examined, using the experimental techniques described by Selye and Bois ("Fourth Annual Report on Stress," Acta Inc., M d. Publ., Montreal, 1954, pp. 533–552). Estradiol caused more intense catabolism, lymphatic-tissue involution and inhibition of inflammation in male than in female Sprague-Dawley rats. This difference was even more evident in adrenalectomized rats maintained on cortisol (400 μg./day), alone or in combination with DCA (100 μg./day). Ovariectomy increased the cortisol-sensitivity of adrenalectomized females to the level of the adrenalectomized male, but progesterone (2 mg./day) decreased it again to the female level. In adrenalectomized (or adrenalectomized-ovariectomized) females, maintained exclusively on DCA (100 μg./day), estradiol did not significantly affect body-weight or inflammation, yet it caused severe thymicolymphatic atrophy. It is concluded that folliculoids do not exert any marked direct effect upon inflammation and anabolism, but act in this respect mainly as "conditioning agents" for antiphlogistic corticoids, such as cortisol; on the other hand, their ability to produce thymico-lymphatic atrophy is direct and does not depend upon the simultaneous presence of cortisol-like compounds in the body.

138. The Effect of Various Pre-Anesthetic Medications on Pituitary-Adrenal Function.

E. S. Siker, M.D., E. Lipschitz, M.D. and *R. Klein, M.D.** University of Pittsburgh, Pittsburgh, Pa.

The effects of pre-anesthetic medications on the level of free serum corticoids was investigated. The corticoids were measured by the method of Nelson and Samuels before and after the intravenous administration of morphine, scopolamine, pentobarbital, atropine and physiologic saline solution. The influence of subcutaneous morphine and scopolamine and intramuscular pentobarbital on the response to a standard intravenous ACTH test was measured in another group of subjects. Corticoid levels were also measured at varying intervals after the administration of ether or N_2O-O_2, thiopental-sodium anesthesia in patients divided into two groups based upon whether or not premedication was used. Premedication with varying combinations of these drugs had no effect on the rise of the serum corticoid level seen after anesthesia. Neither morphine, atropine nor pentobarbital inhibited the rise in the level of free serum corticoids following ACTH. The results of the intravenous administration of these agents showed that the only significant ($P < .02$) change was in the patients receiving pentobarbital. Since they did not inhibit the response to ACTH or to the stimulus of anesthesia, these drugs do not affect the ability of the adrenal to respond. Pentobarbital must inhibit the pituitary secretion of ACTH directly, or the central nervous system centers controlling pituitary ACTH secretion. The great variability found with morphine and scopolamine in the doses used, and the consistency with pentobarbital, suggest that the latter agent may be a more reliable preanesthetic medication for minimizing anxiety or the reaction to anxiety.

139. Effects of Chronic Administration of Meticorten and Meticortelone.

Sibylle Tolksdorf, Ph.D. and *Preston L. Perlman, Ph.D.** Biological Research Laboratories, Schering Corp., Bloomfield, N. J.

Meticorten (Δ^{14}-pregnadiene-17α,21-diol-3,11,20-trione) and Meticortelone (Δ^{14}-pregnadiene-11β,17α,21-triol-3,20-dione) (Herzog *et al.: Science* [in press]), have been

found to yield promising results in the treatment of rheumatic diseases (Bunim *et al.* *J.A.M.A.* **157:** 311, 1955) and to possess in animals "glucocorticoid" activity 3–4 times greater than that of cortisone or of hydrocortisone (Perlman, P. L. and Tolksdorf, S.: *Fed. Proc.*, [to be published]). The daily doses of Meticorten and Meticortelone necessary to suppress growth of 160-gram rats were approximately 2 to 3 mg. By comparison, 4 mg./day of cortisone and 2 mg./day of hydrocortisone did not produce a complete plateau in the growth curves. Rats were examined at autopsy at the beginning of treatment, after receiving 16 to 27 daily injections, and sixteen days after cessation of treatment. The mean body and organ weights of the controls, plotted semilogarithmically against time, showed approximate linear increases (liver, kidney, spleen, testes, seminal vesicles and adrenals). Steroid treatment produced varying degrees of involution of the thymus, spleen, adrenals and seminal vesicles, whereas the growth of livers, kidneys and testes was retarded. Thyroids and pituitaries of the treated animals also appeared reduced in weight. Rapid resumption of growth was observed during the recovery period. Profound lymphopenia and an increase in polymorphoneuclear leukocytes were observed in the Meticorten- and Meticortelone-treated groups without consistent changes in the total leukocyte counts. The lympholytic action of cortisone and hydrocortisone was much less pronounced. The changes reverted to normal shortly after cessation of treatment.

140. The Effect of Hypoadrenalism and Desoxycorticosterone Acetate Upon the Response of the Rat to Ouabain.

David Unterman, M.D., Arthur C. DeGraff, M.D. and *Herbert S. Kupperman, M.D.** Department of Therapeutics, College of Medicine, New York University Bellevue Medical Center, New York, N. Y.

The effect of altered adrenal function in the rat upon the responsiveness of the animal to intravenously administered ouabain was studied electrocardiographically, using the string galvanometer. Conductive disturbances followed by cardiac arrest were selected as the criteria for a positive effect. Twenty-one normal and 19 adrenalectomized rats received ouabain intravenously. An additional 17 adrenalectomized rats were given saline intravenously and served as controls. Sixteen trials with ouabain were conducted in 8 rats which had been implanted subcutaneously with pellets of DCA and maintained on saline drinking water. A significantly greater sensitivity to ouabain was observed in the adrenalectomized animals (positive effect in 11 of the 19, or 58 per cent) as compared with the normal controls (positive effect, 9.5 per cent). In the DCA-implanted rats the incidence of positive effect was 31 per cent, which was higher than that of the control group. Although this difference is not significant, it should be noted that the average dose of ouabain per unit of body weight for the DCA-implanted rats was approximately one-third less than that of the normal controls. The increased sensitivity of the adrenalectomized rat to ouabain is not readily explained in terms of the known changes in potassium metabolism in hypoadrenal states.

141. Inhibitory Action of Hydrocortisone Acetate, 9α-Fluoro-Hydrocortisone Acetate, and ACTH on Estradiol-17β-Induced Uterine Growth.

*Joseph T. Velardo, Ph.D.** and *Somers H. Sturgis, M.D.** Department of Surgery Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

Previously, Velardo, Hisaw and Bever (*Anat. Rec.* **117:** 552, 1953) reported that cortisone acetate and DCA inhibited the uterine growth-promoting action of estradiol. It

thus became of interest to ascertain the action of purified hydrocortisone acetate (compound F acetate) and ACTH in the response of the uterus of the rat to estradiol. 9α-Fluorohydrocortisone acetate was likewise tested against estradiol. Virgin albino rats, 100 days of age, were bilaterally ovariectomized, and one week after castration 0.1 μg. of estradiol was injected subcutaneously daily for three days with each of the hormones previously named. All compounds were injected at separate sites. Seventy-two hours after the initial injection, the rats were killed and the uteri were weighed. These results indicate that compound F acetate was more effective than cortisone acetate in inhibiting estradiol. Concurrent injections of 0.1 μg. of estradiol and 9α-fluorohydrocortisone acetate (0.025 to 0.500 mg. daily) resulted in approximately 10 to 90 per cent inhibition of estradiol-induced uterine growth. Considerably more compound F acetate (0.3 to 2.5 mg. daily) was required for nearly comparable inhibition. ACTH (0.1 to 0.5 mg. daily) produced 35 to 50 per cent restriction of uterine growth, but was without effect in estradiol-treated ovariectomized, adrenalectomized rats.

142. Achard-Thiers Syndrome Due to Bilateral Adrenocortical Hyperplasia: Diagnosis and Treatment.

*M. J. Whitelaw, M.D.** and *D. Island, B. S.* Department of Obstetrics and Gynecology, and Metabolic Unit for Research in Arthritis and Allied Diseases, University of California School of Medicine, San Francisco, Calif.

A 54-year-old female had a two-month sequential history of marked changes in mood, polydipsia, polyuria, weakness, and a 20-pound weight loss. Following diagnosis of diabetes mellitus, brown pigmentation and a beard appeared. A dose of 200 units of NPH insulin daily was required for partial control. Outstanding findings were: a blood pressure of 152/100, a pulse rate of 120, diffuse dark-brown pigmentation of the skin, some hematomata, and a coarse black beard; no striae, no fat pads, and no enlargement of the clitoris. The patient was confused and had paranoid delusions. Laboratory findings: fasting blood sugar level, 236 mg. per 100 ml.; 4+urine sugar; no acetone; a hypochloremic alkalosis; WBC 16,000, with P 96 per cent, L 3 per cent, Eos. 0, by direct count. X-ray examination revealed diffuse demineralization. Urinary steroid studies showed control values of 92 mg. per day of 17-hydroxycorticoids (normal = 8 ± 4), and 33 mg. per day of 17-ketosteroids (normal = 10 ± 5), which ACTH stimulation failed to raise. Following failure to demonstrate a mass by extraperitoneal pneumography, one and two thirds of the hyperplastic adrenals were removed. Six months later the laboratory findings were unremarkable, except for mild fasting hyperglycemia. The beard and excessive pigmentation had disappeared.

143. Effects of Sonic Vibration on Steroids.

Herbert H. Wotiz, Ph.D. and *Thomas C. Smith, Ph.D.** Boston University School of Medicine, Boston, Mass.

Attempts at dispersing steroids into aqueous media for purposes of metabolic studies by existing methods have been hampered by the poor solubility of these compounds. Although ethanol and propylene-glycol may help solve this problem, they affect tissue metabolism. Similarly, sera have recently been found to show 17-dehydrogenase activity. Exposure of estradiol-17β, testosterone, progesterone, and cortisone mixed with distilled water, in glass tubes to 10KC vibration from a raytheon magnetostriction oscillator for one hour produced a fine suspension. Immediately after sonication the steroids were extracted with ethylacetate. An aliquot of each steroid was paper chromatographed

in an appropriate system, and the residual steroid measured by its absorption in the ultraviolet region. Recoveries of the different steroids varied from 85 to 105 per cent. Companion chromatograms of each steroid, stained to detect possible breakdown products, showed only starting material. Infrared spectra of the sonicated steroids showed absorption bands identical with those of the original compounds.

CARBOHYDRATE METABOLISM AND DIABETES

144. Hormonally Induced Changes in Glucose-6-Phosphatase Activity in Rat Liver.

James Ashmore, Ph.D., F. B. Nesbett, M.A. and *A. Baird Hastings, Ph.D.* (Introduced by Billy Baggett, Ph.D.). Harvard Medical School, Boston, Mass.

It has previously been demonstrated that fasting and alloxan diabetes both cause an increase in the activity of rat liver glucose-6-phosphatase (Ashmore, Hastings and Nesbett: *Proc. Nat. Acad. Sci.* **40**: 673, 1954). These studies have been extended to consider the *in vivo* influence of cortical and hypophyseal hormones on this enzyme. The increased phosphatase activity of diabetics is returned to normal by adrenalectomy. Injection of cortical hormone into adrenalectomized diabetic animals restores the phosphatase activity to diabetic levels. Injection of insulin over a period of twenty-four to forty-eight hours results in a decrease of liver glucose-6-phosphatase activity in normal diabetic, adrenalectomized and hypophysectomized animals. Twelve to twenty-four hours are required for pronounced changes in glucose-6-phosphatase activity. No *in vitro* hormone effects on liver have been demonstrated. These results suggest that the physiologically optimum level of activity of liver glucose-6-phosphatase, per gram of liver, is maintained by a balance between insulin and glucocorticoids.

145. Prevention or Abolition of Growth Hormone Diabetes in Hypophysectomized Dogs by Adrenocortical Hormones.

R. C. de Bodo, M.D., N. Altszuler, Ph.D.* and *S. P. Kiang, M.S.* Department of Pharmacology, New York University College of Medicine, New York, N. Y.

Administration of growth hormone (1 mg./Kg./day) to hypophysectomized dogs raises the postabsorptive blood sugar level, abolishes insulin hypersensitivity, produces a diabetic type of glucose tolerance, and causes severe toxic manifestations. Administration of cortisone or hydrocortisone without growth hormone (in dosages of 0.8 to 1.2 mg./Kg./day) to hypophysectomized dogs: a) raises the postabsorptive blood sugar level somewhat, b) ameliorates insulin hypersensitivity, c) diminishes the secondary hypoglycemia of the glucose tolerance test, and d) restores to normal the disposal of an intravenous glucose load. The addition of growth hormone to the steroid regimen enhances the steroid effects listed under a), b) and c), but does not alter that under d), *i.e.*, does not produce the characteristic diabetic glucose tolerance. Thus, the C-11,17-oxycorticosteroids act synergistically with growth hormone in certain respects [a), b), c)], but act also antagonistically in others, as shown by their prevention of the development of growth-hormone diabetes and toxicity. Furthermore, the diabetes produced by growth hormone can be abolished by the addition of the steroids to the growth-hormone regimen. The beneficial effect of the steroids may be related to their ability to enhance the disposal of glucose.

146. Glucose Uptake by White Blood Cells of Patients With Diabetes Mellitus.

*Mary E. Dumm, Ph.D.** Department of Medicine, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Glucose uptake by white blood cells from patients with diabetes mellitus and from nondiabetic human subjects has been measured under aerobic and anaerobic conditions. The diabetic subjects omitted insulin the previous evening and fasting blood samples were taken. The white blood cells were prepared by sedimentation of the erythrocytes at room temperature, as described by Tullis (1953). Total white cell counts and differentials were performed on the original blood and on the suspension of cells studied. Glucose was added to the blood to bring its concentration to more than 200 mg. per 100 ml., and ascorbic acid to a concentration of more than 1 mg. per 100 ml. The cell suspensions were placed in small flasks, under O_2 or N_2, and shaken in a water bath at 37°C. The concentration of glucose was measured at intervals for a period of about four hours. Glucose uptake by diabetic white cells was lower than that by nondiabetic white cells, both aerobically and anaerobically. Insulin (0.1 unit per ml.) further increased aerobic glucose uptake by the cells of the normal subjects and had variable effects on glucose uptake by cells of the diabetic patients.

147. Prolonged Administration of Vitamin B_{12}, Folic Acid and Calcium Pantothenate in Patients With Diabetes Mellitus.

Elaine P. Ralli, M.D., F. Xavier Barbosa, M.D., Mary E. Dumm, Ph.D., Edith M. Beck, M.D.* and *Bertram Laken, M.S.* Department of Medicine, College of Medicine, New York University-Bellevue Medical Center, New York, N. Y.

Diabetic patients of different ages who had been observed in a diabetic clinic for several years were the subjects of this study. Blood determinations were made before and during a period of treatment with vitamin B_{12}, vitamin B_{12} plus folic acid or calcium pantothenate. The blood concentrations of the following were determined: serum protein by paper electrophoresis, plasma and whole blood sulfhydryl, blood glucose and ascorbic acid, and serum cholesterol. In some of the patients insulin sensitivity tests were performed before and after the administration of the supplement. The study dealt with the effect of these supplements on the course of the disease, the insulin requirement and blood determinations. The administration of vitamin B_{12} was not associated with any striking change in the plasma or nonprotein sulfhydryl levels (glutathione). Some patients showed an increased sensitivity to insulin following vitamin B_{12} administration. The administration of calcium pantothenate was in some cases associated with a decrease in the fasting levels of blood glucose. The results are of interest, in view of the suggestion by certain authors that there is a relationship between the levels of glutathione and of blood glucose.

148. Effect of Prolonged Growth-Hormone Administration on Pancreatic Alpha Cells in Hypophysectomized Rats.

*Bruno W. Volk, M.D.** and *Martin G. Goldner, M.D.** Jewish Chronic Disease Hospital, Brooklyn, New York, N. Y.

The diabetogenic effect of anterior-pituitary extract has repeatedly induced investigations on the possible direct action of the hormones of the adenohypophysis on the pancreatic islets. Since the experimental results are largely contradictory, we undertook to study the effect of purified growth hormone on the morphology of the islets, the alpha cells and the alpha/beta cell ratio of the pancreatic islets of hypophysectomized rats. The daily subcutaneous administration of 1 mg./200 Gm. body weight for three weeks resulted in hyperplasia of the islets with significant increase of the alpha/beta cell ratio. This was observed in serial sections, using alternatingly Gomori's technique and a newly

elaborated modification of the Davenport's silver impregnation procedure. Daily injection of similar amounts of growth hormone for six weeks produced an absolute and relative decrease of the alpha cells. The remaining alpha cells and most beta cells showed marked degranulation. None of the animals became diabetic. In untreated hypophysectomized rats allowed to survive up to four and a half months after operation, the sizes of the islets, the alpha cells and the alpha/beta cell ratio were unchanged.

THE SEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE INDIANA UNIVERSITY SCHOOL OF MEDICINE AT THE CONTINUATION STUDY CENTER OF THE INDIANA UNIVERSITY MEDICAL CENTER, INDIANAPOLIS, INDIANA

September 26–October 1, 1955

The faculty will consist of twenty-one eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Post Graduate Office, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis 7, Indiana. Registration is limited to 100; tuition fee is $100.00. Arrangements for housing accommodations will be made through the Indiana Medical Center.

FACULTY

Willard M. Allen, M.D.,Professor of Obstetrics & Gynecology, Washington University School of Medicine, Saint Louis, Missouri.

Brown M. Dobyns, M.D., Associate Professor of Surgery, Western Reserve School of Medicine, Cleveland, Ohio.

Ralph I. Dorfman, Ph.D., Associate Director, Worcester Foundation, Shrewsbury, Mass., and Research Professor of Biochemistry, Boston University School of Medicine, Boston, Mass.

Frank L. Engel, M.D., Associate Professor of Medicine, Assistant Professor of Physiology, Duke University, Durham, N.C.

Earl T. Engle, Ph.D., Professor of Anatomy, Department of Obstetrics & Gynecology, Columbia University, College of Physicians and Surgeons, New York, N. Y.

Roberto F. Escamilla, M.D., Associate Clinical Professor of Medicine, University of California School of Medicine, San Francisco, California.

Sprague H. Gardiner, M.D., Associate Professor of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, Indiana.

Robert A. Garrett, M.D., Professor of Urology, Indiana University School of Medicine. Indianapolis, Indiana.

Robert B. Greenblatt, M.D., Professor of Endocrinology, Medical College of Georgia, Augusta, Georgia.

Carl P. Huber, M.D., Chairman, Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, Indiana.

Glenn W. Irwin, M.D., Assistant Professor of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

Charles W. Lloyd, M.D., Assistant Professor of Obstetrics and Assistant Professor of Medicine, State University of New York, Upstate Medical Center, Syracuse, N. Y.

Karl E. Paschkis, M.D., Associate Professor of Medicine; Associate Professor of Physiology; Director, Division of Endocrine and Cancer Research, The Jefferson Medical College of Philadelphia, Philadelphia, Pa.

Franklin B. Peck, Sr., M.D., Associate Professor of Medicine, Indiana University School of Medicine; Director, Medical Research Cooperation, Lilly Research Laboratories, Indianapolis, Indiana.

Rulon W. Rawson, M.D., Professor of Medicine, Sloan-Kettering Division, Cornell University Medical School, New York, N. Y.

Edward C. Reifenstein, Jr., M.D., Associate Medical Director, E. R. Squibb & Sons, Division of Olin Mathieson Chemical Corporation; Assistant Clinical Professor of Medicine, New York Medical College, New York, N. Y.

Edward H. Rynearson, M.D., Chairman of Sections, Mayo Clinic, Rochester, Minnesota.

Charles E. Test, M.D., Assistant Professor of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

Henry H. Turner, M.D., Clinical Professor of Medicine, University of Oklahoma School of Medicine; Chief, Endocrine Clinic, University Hospitals, Oklahoma City, Oklahoma

Lawson Wilkins, M.D., Associate Professor of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Don E. Wood, M.D., Associate Professor, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana.

PROGRAM OF COURSE

Monday, September 26, 1955

8:00 to 8:45 A.M. Registration: Place—*Lobby, Indiana State Board of Health Building*

Introductions: Dr. John D. Van Nuys, Dean, Indiana University School of Medicine

Dr. Don E. Wood, Chairman of Local Committee on Arrangements

Dr. Roberto F. Escamilla, Chairman of Postgraduate Committee of the Endocrine Society

Chairman of morning session—Dr. Don E. Wood

9:00 to 9:45 A.M. Anterior pituitary: anatomy and present status of physiology; relation to hypothalamus—*Dr. Charles W. Lloyd*

9:45 to 10:30 A.M. Hypopituitarism: dwarfism, Simmonds' disease, Sheehan's syndrome, Froelich's syndrome—*Dr. Roberto F. Escamilla*

10:30 to 10:45 A.M. Intermission

10:45 to 11:30 A.M. Hyperpituitarism: gigantism and acromegaly—*Dr. Karl E. Paschkis*

11:30 to 12:00 N. Posterior pituitary and intermediate lobe anatomy and physiology: relation to hypothalamus—*Dr. Charles W. Lloyd*

12:00 to 12:30 P.M. Diabetes insipidus: role of posterior pituitary in fluid balance and hypertension—*Dr. Charles W. Lloyd*

12:30 to 2:00 P.M. Lunch

Chairman of afternoon session—Dr. Karl E. Paschkis

2:00 to 2:30 P.M. Examination of the patient for endocrine disease—*Dr. Frank L. Engel*

2:30 to 3:00 P.M. Place of the laboratory in endocrine diagnosis—*Dr. Roberto F. Escamilla*

3:00 to 3:15 P.M. Intermission

3:15 to 4:30 P.M. Demonstration of laboratory procedures—*Dr. Robert B. Greenblatt and Dr. Don E. Wood*

4:30 to 5:00 P.M. Question and answer period

6:30 P.M. Get-acquainted party. Cocktails and dinner for faculty and students, sponsored by Eli Lilly & Company, at Indianapolis Athletic Club

Tuesday, September 27, 1955

Chairman of morning session—Dr. Frank L. Engel

9:00 to 9:30 A.M. Thyroid: anatomy and physiology; relation to pituitary—*Dr. Brown M. Dobyns*

9:30 to 10:00 A.M. Goiter: classification and present concepts—*Dr. Rulon W. Rawson*

10:00 to 10:40 A.M. Hypothyroidism in childhood and in adults—*Dr. Henry H. Turner*

10:40 to 10:50 A.M. Intermission

10:50 to 11:30 A.M. Hyperthyroidism—*Dr. Rulon W. Rawson*

11:30 to 12:00 N. Thyroid cancer—*Dr. Brown M. Dobyns*

12:00 to 12:30 P.M. Thyroiditis—*Dr. Rulon W. Rawson*

12:30 to 2:00 P.M. Lunch

Chairman of afternoon session—Dr. Rulon W. Rawson

2:00 to 2:30 P.M. Parathyroids: anatomy and physiology—*Dr. Edward C. Reifenstein, Jr.*
2:30 to 3:15 P.M. Hypoparathyroidism—*Dr. Glenn W. Irwin*
3:15 to 3:30 P.M. Intermission
3:30 to 4:15 P.M. Hyperparathyroidism—*Dr. Frank L. Engel*
4:15 to 4:45 P.M. Metabolic bone disease: differential diagnosis and treatment—*Dr. Edward C. Reifenstein, Jr.*
4:45 to 5:30 P.M. Question and answer period

Wednesday, September 28, 1955

Chairman of morning session—Dr. Lawson Wilkins

9:00 to 9:45 A.M. Adrenal cortex: anatomy, chemistry and physiology—*Dr. Ralph I. Dorfman*
9:45 to 10:30 A.M. Adrenal insufficiency—*Dr. Frank L. Engel*
10:30 to 10:45 A.M. Intermission
10:45 to 11:30 A.M. Cushing's disease and Cushing's syndrome—*Dr. Robert B. Greenblatt*
11:30 to 12:00 N. Adrenogenital syndrome, including congenital type: diagnosis and treatment—*Dr. Lawson Wilkins*
12:00 to 12:30 P.M. Other adrenal cortical tumors; aldosteronism—*Dr. Karl E. Paschkis*
12:30 to 2:00 P.M. Lunch

Chairman of afternoon session—Dr. Ralph I. Dorfman

2:00 to 2:45 P.M. Hermaphroditism: classification and diagnosis; feminizing adrenal tumors; selection of sex of rearing; treatment—*Dr. Lawson Wilkins*
2:45 to 3:30 P.M. Use of cortisone, corticotropin and related substances: management of patient before and after adrenal surgery—*Dr. Frank L. Engel*
3:30 to 3:45 P.M. Intermission
3:45 to 4:30 P.M. Hyperinsulinism and spontaneous hypoglycemia—*Dr. Edward H. Rynearson*
4:30 to 5:00 P.M. Obesity and leanness—*Dr. Edward H. Rynearson*
5:00 to 5:30 P.M. Question and answer period

Thursday, September 29, 1955

Chairman of morning session—Dr. Edward C. Reifenstein, Jr.

9:00 to 9:30 A.M. The testis: anatomy and physiology—*Dr. Earl T. Engle*
9:30 to 10:15 A.M. Male hypogonadism—*Dr. Henry H. Turner*
10:15 to 10:30 A.M. Intermission
10:30 to 11:00 A.M. Cryptorchidism—*Dr. Robert Garrett*
11:00 to 11:30 A.M. Male climacteric; impotence—*Dr. Henry H. Turner*
11:30 to 12:15 P.M. Testicular tumors, including male pseudohermaphroditism—*Dr. Robert Garrett*

12:15 to 2:00 P.M. Lunch

Chairman of afternoon session—Dr. Willard M. Allen

2:00 to 2:30 P.M. Hormones and cancer; present knowledge—*Dr. Ralph I. Dorfman*
2:30 to 3:00 P.M. Male infertility: present status of therapy—*Dr. Karl E. Paschkis*
3:00 to 3:30 P.M. Gynecomastia; Klinefelter's syndrome—*Dr. Don E. Wood*
3:30 to 3:45 P.M. Intermission
3:45 to 5:00 P.M. Case presentations: pituitary, thyroid, parathyroids, adrenals, testes—*Dr. Don E. Wood, and resident physicians,* Indiana University Medical Center
5:00 to 5:30 P.M. Questions and answers

Friday, September 30, 1955

Chairman of morning session—Dr. Robert B. Greenblatt

9:00 to 9:45 A.M. The ovary: anatomy and physiology—*Dr. Earl T. Engle*
9:45 to 10:45 A.M. Ovarian hypofunction syndromes, including female eunuchoidism, ovarian aplasia, Turner's syndrome—*Dr. Henry H. Turner*
10:45 to 11:00 A.M. Intermission
11:00 to 11:30 A.M. Ovarian tumors of endocrine significance—*Dr. Carl P. Huber*
11:30 to 12:30 P.M. Functional ovarian disorders, including meno- and metrorrhagia—*Dr. Willard M. Allen*

12:30 to 2:00 P.M. Lunch

Chairman of afternoon session—Dr. Earl T. Engle

2:00 to 2:30 P.M. The menopause—*Dr. Willard M. Allen*
2:30 to 3:00 P.M. Female infertility—*Dr. Robert B. Greenblatt*
3:00 to 3:15 P.M. Intermission
3:15 to 4:30 P.M. Endocrine problems of pregnancy—*Dr. Sprague H. Gardiner*
4:30 to 5:30 P.M. Case presentations: ovaries, Questions and answers—*Dr. Don E. Wood, and resident physicians,* Indiana University Medical Center
6:30 P.M. Banquet of the Course, including students and faculty. Cost $4.00. Tickets will be sold at time of registration. *Room M-124, Student Union Bldg., Indiana University Medical Center*

Speaker: *Dr. Carl P. Huber*—"Stress during labor"

Saturday, October 1, 1955

Chairman of the morning session— Dr. Roberto F. Escamilla

9:00 to 9:30 A.M. Simple hirsutism; Stein-Leventhal syndrome—*Dr. Willard M. Allen*
9:30 to 10:15 A.M. Adrenal medulla; pheochromocytoma—*Dr. Glenn W. Irwin*
10:15 to 10:30 A.M. Intermission.
10:30 to 10:45 A.M. Chemistry and actions of available insulins—*Dr. Franklin B. Peck, Sr.*
10:45 to 12:00 N. Essentials in management of uncomplicated diabetes—*Dr. Charles E. Test*
12:00 to 12:30 P.M. Pointers in management of complications of diabetes—*Dr. Franklin B. Peck, Sr.*

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.

2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

(*For Recipients of the 1955 Awards see pages 906–910*)

RECIPIENTS OF 1955 AWARDS

JOSEPH MERANTE

DR. CARL R. MOORE

THE MEDAL OF THE ENDOCRINE SOCIETY

THE MEDAL OF THE ENDOCRINE SOCIETY

Dr. Carl R. Moore has been selected as the recipient of the Medal of The Endocrine Society for 1955 in recognition of his distinguished achievements in, and contributions to endocrinology.

Dr. Moore was born in Brookline, Missouri, in 1892 and attended Drury College in Missouri, receiving his bachelor's degree there in 1913 and master's degree in 1914. He then attended the University of Chicago, where he received his Ph.D. degree in 1916 for work on a problem in fertilization. He has remained there since, rose through the ranks of the Department of Zoology, became a full professor in 1928, and chairman of the department in 1934.

As a student of Frank R. Lillie, Dr. Moore has sustained and enlarged a great tradition. Through him, the biological meaning of the descent of the testes was learned, demonstrating that seminiferous epithelium in many forms could not develop well in the warmth of the abdomen. By elaborate work on the genital accessories, he has provided descriptions of the operations of androgens on these receptors and so characterized the reparative force, first of early crude extracts of the testis, and later of pure chemicals. Through him, it has been learned that much of the antagonism between gonads of opposite sexes is exerted by hormones through the pituitary body, and not directly. Inspired early in his career to inquire into the endocrine mechanisms entering into the control of fetal development, Dr. Moore has, in later dexterous work on the opossum, advanced the understanding of this obscure and difficult problem.

Dr. Moore is a Past-President of The Endocrine Society, retaining the office for two terms—from 1944 to 1946.

THE CIBA AWARD

PHILIP J. FLEURY

DR. JACK GROSS

The recipient of The Endocrine Society Ciba Award for 1955 is *Dr. Jack Gross*, Associate Professor of Anatomy at the College of Medicine, State University of New York, Brooklyn, N. Y.

The scientific career of Dr. Gross may be divided into three parts, which he pursued in Canada, England and the United States, respectively.

In Canada, Dr. Gross obtained his medical and scientific education at McGill University. During his medical training, he was known as the student who spent his evenings and week-ends teasing the thyroid gland in various ways. After a period of active service with the Canadian Army Medical Corps, Dr. Gross went

(*Continued on next page*)

DR. WILLIAM G. ROBERTSON

AYERST, McKENNA AND HARRISON FELLOWSHIP

Dr. William G. Robertson, the recipient of the Ayerst, McKenna and Harrison Fellowship of The Endocrine Society for 1955, is a native of New Jersey and received his baccalaureate training at Rutgers University, where he also will complete his work for the Ph.D. degree this year. In contrast to so many experimental endocrinologists, who use small animals for their work, Dr. Robertson has found his best experimental material in one of the largest—the dairy cow.

His particular studies, carried out under the direction of Dr. J. P. Mixner, have been concerned with the blood levels of 17-hydroxycorticosterones in this species. From his work it would appear that a condition in dairy cattle usually referred to as "ketosis" has some relation to the blood level of these hormones. Dr. Robertson intends to use the opportunity afforded by this Fellowship in further study of a problem that has both academic and practical interest.

back to McGill as a Ph.D. student and lecturer in the Department of Anatomy. Shortly, he acquired skill in the use of radio-elements and began to devise new ingenious applications of these to the solving of old problems. Working in close association with Dr. C. P. Leblond, he traced parenterally administered radio-thyroxine into the bile as well as into feces and demonstrated that free thyroxine is present in thyroid tissue in a concentration sufficient to establish a gradient between this tissue and plasma, such as to insure its passsage to the circulation. Incidentally, he noted that, besides thyroxine, one other iodinated substance only was present in both thyroid and plasma, the enigmatic Compound No. 1.

The lure of this unknown substance took him to Sir Charles Harington's laboratory in England where, working in close association with Dr. R. Pitt-Rivers, he isolated Compound No. 1 in pure form. When enough had been isolated, he was able to identify this substance as 3:3′:5′-triiodothyronine, synthesize it from diiodothyronine and show it to be several times more active than thyroxine in both experimental animals and man.

In the third and current phase of his career, Dr. Gross is working in New York, where he studies both conditions of production and utilization of triiodothyronine. His recent work suggests that thyroxine is the hormone secreted by the gland but that triiodothyronine is the peripherally active principle resulting from thyroxine's deiodination. He is now engaged in exploring the possibilities of this stimulating concept.

Dr. Gross' work is characterized by a sense of balance and a feeling of completeness. Both characteristics are also those of his personality. It is hoped that his work on triiodothyronine will be only the first step in a brilliant endocrinological career.

DR. ROBERT O. SCOW

THE SCHERING SCHOLAR

Dr. Robert O. Scow has been selected as the Schering Scholar of The Endocrine Society for 1955.

Dr. Scow is an Investigator in Basic Research at the National Institutes of Health, Bethesda, Maryland, and has elected to spend this year at the Institute of Professor B. A. Houssay in Buenos Aires. He proposes to study the effects of hormonal and nutritional factors on the production, prevention and cure of experimental diabetes.

He received his earlier training, as well as an M.D. degree, at the University of California. He has published a considerable number of papers on the thyroid, in particular on the effects of thyroidectomy in very young animals.

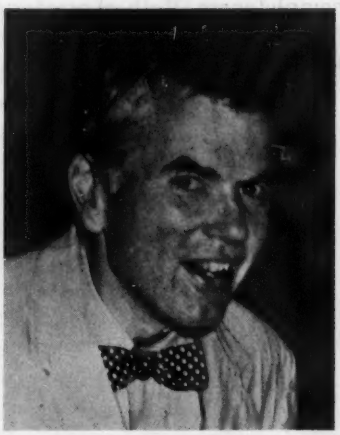
Dr. Scow has already left the country for Argentina. He has taken his family with him and, as his three children range in age from 8 months to 4½ years, there should be an added word of appreciation of Mrs. Scow's sacrifices for the advancement of science.

THE UPJOHN SCHOLAR

The Upjohn Scholar of The Endocrine Society for 1955 is *Dr. Fuller Albright* of the Massachusetts General Hospital and Harvard University.

It is not necessary to add anything regarding Dr. Albright's many great contributions to endocrinology, which have been previously recognized by his selection for the Squibb Award of the Society in 1948 and by his election as President of the Society in 1946.

Dr. Albright will spend the year visiting and working in laboratories and clinics in Europe.



DR. FULLER ALBRIGHT